SHOUT
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IT

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

Editorial Staff

E. G. CONKLIN—*Princeton University.*
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Managing Editor

FRANK R. LILLIE—*The University of Chicago.*

VOLUME XXXIII.

WOODS HOLE, MASS.
JULY TO DECEMBER, 1917

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BIOLOGICAL BULLETIN

THE MARINE BIOLOGICAL LABORATORY

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I. TRUSTEES

AUGUST, 1916

EX OFFICIO

FRANK R. LILLIE, *Director*, The University of Chicago.
GILMAN A. DREW, *Assistant Director*, Marine Biological Laboratory.
D. BLAKELY HOAR, *Treasurer*, 161 Devonshire St., Boston, Mass.
GARY N. CALKINS, *Clerk of the Corporation*, Columbia University.

TO SERVE UNTIL 1920

H. H. DONALDSON........Wistar Institute of Anatomy and Biology.
M. J. GREENMAN.........Wistar Institute of Anatomy and Biology.
C. W. HARGITT..........Syracuse University.
H. S. JENNINGS..........Johns Hopkins University.
GEORGE LEFEVRE........University of Missouri, *Secretary of the Board.*

A. P. MATHEWS	The University of Chicago.
G. H. PARKER	Harvard University.
HENRY B. WARD	University of Illinois.

TO SERVE UNTIL 1919

H. C. BUMPUS	Tufts College.
R. A. HARPER	Columbia University.
W. A. LOCY	Northwestern University.
JACQUES LOEB	The Rockefeller Institute for Medical Research.
F. P. MALL	Johns Hopkins University.
GEORGE T. MOORE	Missouri Botanical Garden, St. Louis.
L. L. NUNN	Telluride, Colo.
JOHN C. PHILLIPS	299 Berkeley Street, Boston, Mass.

TO SERVE UNTIL 1918

CORNELIA M. CLAPP	Mount Holyoke College.
E. G. CONKLIN	Princeton University.
ROSS G. HARRISON	Yale University.
CAMILLUS G. KIDDER	27 William Street, New York City.
M. M. METCALF	Oberlin, Ohio.
WILLIAM PATTEN	Dartmouth College.
JACOB REIGHARD	University of Michigan.

TO SERVE UNTIL 1917

S. F. CLARKE	Williamstown, Mass.
CHARLES A. COOLIDGE	Ames Building, Boston, Mass.
C. R. CRANE	Woods Hole, Mass., *President of the Board.*
ALFRED G. MAYER	Carnegie Institution.
C. E. MCCLUNG	University of Pennsylvania.
T. H. MORGAN	Columbia University.
ERWIN F. SMITH	United States Department of Agriculture.
E. B. WILSON	Columbia University.

II. ACT OF INCORPORATION

NO. 3170.

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, Henry B. Pierce, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the Marine Biological Laboratory, with the powers, rights and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand, Eight Hundred and Eighty-Eight.

HENRY B. PIERCE,
(Seal). *Secretary of the Commonwealth.*

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio*, members of the Board of Trustees, and Trustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio*.

II. Special meetings of the members may be called by the Trustees, to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members of the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee on finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution on such terms as may then be agreed upon.

IX. These By-Laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-Laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE TREASURER'S REPORT

STATEMENT OF AUDIT

February 28, 1917.

MR. D. BLAKELY HOAR,
161 Devonshire Street,
Boston.

Dear Sir: We have completed our audit of the accounts of the Marine Biological Laboratory for the year ended December 31, 1916, as kept both at your office in Boston and at Woods Hole and report thereon in the accompanying exhibits and schedules:

Exhibit *A*—Balance-Sheet as of December 31, 1916.
B—Income-and-Expense for Year ended December 31, 1916.
Schedule I—Investments (Book Values).
II—Cash Receipts and Payments on Account of Investments.
III—Summary of Inventory of Land, Buildings, and Equipment.
III*a*—Inventory of Land, Buildings, and Equipment, January 1, 1916.
IV—Balancing Account.

We certify that subject to the comments herewith the balance. sheet and income-and-expense statement shown herewith in Exhibits *A* and *B* are in accordance with the books and correct to the best of our knowledge and belief.

Very respectfully,
HARVEY S. CHASE & COMPANY,
Certified Public Accountants.

Note: Schedules II–IV are omitted in the following reports.

MARINE BIOLOGICAL LABORATORY BALANCE-SHEET, DECEMBER 31, 1916

Assets

Cash in Bank	$ 3,322.96	
Petty Cash at Woods Hole	200.00	$ 3,522.96
Accounts receivable		5,942.01
Investments (Schedule I)	$ 11,086.01	
Investment cash (Schedule II)	678.54	11,764.55

Inventories (not available)		
Plant account (Schedule III):		
Land	$ 78,406.29	
Buildings	163,848.88	
Equipment	73,303.36	
	$315,558.53	
Less reserve for depreciation	6,942.12	308,616.41
		$329,845.93

Liabilities and Surplus

Accounts payable		$ 90.58
Suspense items		3.26
Notes payable	$ 3,000.00	
Turst funds	8,764.55	11,764.55
Mortgage on Gansett property		16,409.82
Balancing account		301,577.72
		$329,845.93

MARINE BIOLOGICAL LABORATORY INCOME AND EXPENSE FOR YEAR ENDED DECEMBER 31, 1916

	Expense	Income	Loss	Gain
Administration expense	$ 6,923.92		$ 6,923.92	
BIOLOGICAL BULLETIN	2,679.84	$ 1,534.08	1,145.76*	
Boat department expense	5,023.09		5,023.09	
Carpenter department	781.98	82.02	699.96	
Chemical department expense	1,455.45		1,455.45	
Dormitories	1,667.98	1,550.80	117.18	
Fish trap	1,348.68	1,406.92		$ 58.24
Interest on notes payable	150.00		150.00	
Instruction	4,075.70	5,075.00		999.30
Lectures, philosophical	100.00		100.00	
Lectures, evening	52.80		52.80	
Library department	3,447.37		3,447.37	
Mess	14,737.68	14,636.16	101.52	
Membership dues		980.00		980.00
Maintenance, buildings and grounds	6,180.18		6,180.18	
New Laboratory expense	2,636.11		2,636.11	
Pumping station expense	542.01		542.01	
Research income		3,650.00		3,650.00
Sundry expense and income	1,239.12	2,765.24		1,526.12
Supply department	13,838.43	21,096.65		7,258.22
Special dormitory expense, Dexter House	1,122.31		1,122.31	
Total expense	$68.002.65	$52,776.87	$29,697.66	$14,471.88
Total income	52,776.87		14,471.88	
Excess of expense	$15,225.78		$15,225.78	
Contribution by Mr. C. R. Crane	20,000.00			
Excess carried to balancing account	$ 4,774.22			

* Membership dues which are credited to the BIOLOGICAL BULLETIN reduce the actual deficit to $165.76.

Marine Biological Laboratory Investments (Book Values), December 31, 1916

Reserve Fund

$3,000 American Telephone & Telegraph Company, 4's (represented by receipts for same as collateral)	$2,921.25	
3/4 of $500 Western Telephone & Telegraph Company's 5's	372.66	
6 shares American Smelting & Refining Company Preferred (represented by receipts for same as collateral)	732.00	
8 shares General Electric Company	972.05	
14 shares United Shoe Machinery Company Preferred	393.75	
5 shares Massachusetts Gas Companies, Preferred	444.63	$ 5,836.34

Lucretia Crocker Fund

1/5 of $1,000 American Telephone & Telegraph Company, 4's	$ 194.75	
18 shares Vermont & Massachusetts Railroad Company	2,416.50	
1 share American Telephone & Telegraph Company	127.06	
1 share West End Street Railway Company	83.00	
2½ shares General Electric Company	323.85	3,145.16

Library Fund

4/5 of $1,000 American Telephone & Telegraph Company, 4's	$ 779.00	
1/4 of $500 Western Telephone & Telegraph Company, 5's	124.22	
3 shares American Telephone & Telegraph Company	381.18	
1 share American Smelting & Refining Company, Preferred	122.00	
2½ shares General Electric Company	288.10	
5 shares United Shoe Machinery Company, Preferred	140.63	
3 shares Massachusetts Gas Companies, Preferred	269.38	2,104.51
Total (Exhibit *A*)		$11,086.01

V. THE LIBRARIAN'S REPORT

August 8, 1916

As compared with last year, all numbers cataloged in the library make a total of 12,615. Of this total the following are new accessions:

Journals and serials from societies, laboratories, etc.	583
Books	84
Reprints	777
Last installment of A. M. N. H. loan	1,125

With the help of Prof. R. W. Tower, librarian of the American Museum of Natural History, who has been exceptionally generous in his coöperation, practically the entire loan has been placed on our shelves. Professor Tower has agreed to send to the library as received the current parts of 32 exchanges listed as follows:

Amer. Phil. Soc. Proc.,
Amsterdam, Verh. K. Akad. Wetenschappen,
Arkiv f. Botanik,
Arkiv f. Zoologi,
Ann. Soc. Zoöl. et Malacol. de Belgique,
Bull. Soc. Roy. de Botan. de Belgique,
Canadian Entomologist,
Bull. Int. Acad. Cracovie,
Dublin, Scientific Proc. Roy. Soc.,
Proc. Roy. Soc. Edinburgh,
Trans. and Proc. Botan. Soc. Edinburgh,
Berichte d. Naturf. Gesell. Freiburg,
Journ. Linnean Soc. London,
Proc. Roy. Soc. London, Ser. B,
Trans. Zoöl. Soc. London,
Mem. and Proc. Manchester Literary and Phil. Soc.,
Trans. and Rep. Manchester Micr. Soc.,
Annales du Mus. d'Hist. Nat. Marseilles,
Medd. K. Vetenskapsakad. Nobelinstitut,
Journ. N. Y. Botan. Gard.,
Bull. Mus. Hist. Nat. Paris,
Atti d. Soc. Toscana Pisa,
Sitzungsber. d. K. B. ges. d. wiss. Math. Natur. Classe, Prag.,
Bull. Acad. Imp. Sci. St. Petersburg,
Memoirs Acad. Imp. Sci. St. Petersburg,
Stettiner Entomol. Zeitung,
K. Svenska Ver. Akad. Aarsbok,
K. Svenska Vet. Akad. Handlingar,
Boll. Mus. Zoöl. Comp. Anat. Torino,
Sitzungsber. Akad. wiss. Math. Naturw. Klasse, Wien,
Verhandlungen K. K. Zool. Bot. Gesell., Wien.

2,269 volumes and parts of volumes have been accessioned. 867 of these were bound when received. In order to care for the remainder, 656 volumes had to be bound this year, which about doubled our usual binding bill, adding to it $667.59. (This with

$171.90 previously expended for a former installment of this loan, makes a total cost for binding of $839.49.) In addition to the above, the American Museum has also given about 1,000 pamphlets that have not been cataloged.

A considerable addition will be made to the library through the sale of duplicate material representing a value of about $350. The details of this will be announced later.

A number of gifts have been made to the library which are greatly valued. From publishers, whose works are especially well placed for examination here, we have received the following:

Open Court Publishing Co., 2 books,
Cambridge University Press, 31 books,
Saunders, 1 book,
University of Chicago Press, 2 books.

Books have been received from authors as follows: A. P. Mathews, C. M. Child, J. M. Coulter, C. F. Chamberlain, M. F. Guyer, F. D. Woods, H. H. Donaldson, Henry Kraemer, C. J. Herrick, J. F. Abbott, H. E. Jordan, J. S. Ferguson, and Ramon y Cajal.

Miss Foot presented a long series of papers by Boveri and Whitman and Dr. F. R. Lillie has given 10 miscellaneous volumes.

777 reprints have been given by a long list of authors, among whom are especially to be mentioned 19 English writers: Wm. Bateson, L. Doncaster, E. S. Goodrich, W. Woodland, A. D. Cotton, W. M. Tattersall, W. E. Shipley, R. Gurney, T. V. Hoggson, Sir Charles Elliot, R. C. Punnett, T. Johnson, Richard Elmhist, J. F. Gemmill, F. Jeffrey Bell, W. B. Benham, A. Willey, W. A. Herdman, and Arnold T. Watson.

Occasion is taken here to thank the other numerous donors whose names may not be given for lack of space.

Among miscellaneous gifts are 16 volumes of the *Biochemische Zeitschrift* from Dr. A. R. Moore, which will be of great value in starting this new set. The Wistar Institute Monographs have been presented by Dr. Greeman, and the Rockefeller Institute has given a set of the Rockefeller Institute Studies. The Surgeon-General's Library has also presented Series 2 of the Index Catalog, which fills a long felt need.

Dr. Drew has deposited with the library his valuable collection of reprints and these have been in constant use this summer.

Under the head of journals and serials from museums, academies, laboratories, etc., the following sets have been completed this year:

Journal of Medical Research,
Zeit. f. Biochem. u. Biophysik,
Kansas University Science Bulletin.

223 volumes have been added during the year to incomplete sets.

The 31 serials listed below were not on our shelves last year:

Univ. Calif. Pub. in Physiology,
Univ. Cal. Pub. in Botany,
Bull. Soc. Roy. de botan. de Belgique,
Mem. Soc. Roy. de Liége,
Bull. Mus. d'hist. nat. Paris,
Abh. Naturwiss. Vereins zu Bremen,
Mem. and Proc. Manchester Literary and Phil. Soc.,
Proc. Roy. Soc. London, Sec. B,
Verhandlungen Zool. Botan. Gesell. Wien,
Sitzungsber. K. Akad. wiss. Wien,
Mem. Soc. Nat. Kiew,
Sitzungsber. Naturforsch. Gesell. Dorpat,
Ann. Mus. Zool. Acad. Imp. Sci. St. Petersburg,
Bull. Acad. Imp. Sci. St. Petersburg,
Mem. Acad. Imp. Sci. St. Petersburg,
Annals Scottish Nat. Hist. Soc.,
Proc. Roy. Soc. Edinburgh,
Sitzungsber. K. B. Gesell. wiss. Prag,
Verh. Natur. wiss. Vereins Heidelberg,
Math. u. Naturwiss. Berichte aus Ungarn,
Berichte d. Naturfor. Gesell. Freiburg,
Verh. K. Akad. Wetensch. Amsterdam,
Proc. Roy. Dublin Soc.,
Atti Soc. Toscana Pisa,
Boll. Mus. Zool. Comp. Anat. Torino,
K. Svenska Vet. Akad. Aarsbok,
K. Svenska Vet. Akad. Handlingar,
Medd. Vet. Akad. Noberinstitut.

We have also entered subscriptions for new journals as follows:

Zeit. f. induk. abstamm. u. vererbungslehre,

Genetics,
Journ. Med. Res.,
Physiological Abstracts,
Biochem. Zeitschrift.

One new exchange with the BIOLOGICAL BULLETIN has been arranged: *Bulletin de la Societé Zool. de France.*

Dr. H. H. Donaldson has generously sent a check to cover the cost of a new Century Dictionary, which has been greatly needed.

Attention is called here to our collection of photographs of foreign laboratories secured by exchange. It is planned to extend this collection to include other laboratories.

It was pointed out last year that four years ago a fund was started to aid us in extending the journal files. Last year the subscriptions to this fund had reached the sum of $91. This year a much larger group of investigators (60) whose names follow, continuing Dr. Tashiro's lead, contributed in amounts from 50 cents to $2.00. A total of $53.00 has been received in this way to date.

W. C. Allee,
Ezra Allen,
F. H. Adler,
G. A. Baitsell,
R. H. Bowen,
C. B. Bridges,
R. A. Budington,
G. L. Carver,
Eleanor Carothers,
C. M. Clapp,
C. M. Child,
C. L. Chapin,
E. J. Cohn,
M. Copeland,
E. V. Cowdry,
I. J. Davies,
G. A. Drew,
D. J. Edwards,
W. E. Garrey,
R. S. Lillie,
Jacques Loeb,
E. J. Lund,
Clara J. Lynch,
E. P. Lyon,
E. E. MacMorland,
C. E. McClung,
T. H. Morgan,
Stewart Mudd,
W. J. V. Osterhout,
Charles Packard,
B. M. Patten,
C. L. Parmenter,
Florence Peebles,
A. Richards,
Miss A. E. Richards,
C. G. Rogers,
F. M. Root,
A. F. Shull,

Wilson P. Gee,
A. J. Goldfarb,
H. B. Goodrich,
A. O. Gross,
M. F. Guyer,
L. V. Heilbrunn,
Mary J. Hogue,
E. R. Hoskins,
J. S. Huxley,
F. R. Lillie,
R. A. Spaeth,
A. H. Sturtevant,
H. D. Taylor,
Clara L. Ware,
M. W. Welch,
D. H. Wenrich,
Paul S. Welch,
H. L. Wieman,
W. H. Weston,
Miss A. E. Woodward.

Dr. Bumpus contributed $25 and Doctors Meigs, A. G. Mayer, and Knower added $10 each toward completing the sums previously promised by them. In this way the total amount of $108 was received this year. In addition there are still a few subscriptions expected from promises already published.

This fund represents a most important coöperative spirit in the development of the library. The library is used more now than ever before, as witnessed by the fact that 350 books were charged in one day. Never before have so many individuals of the Laboratory shown their appreciation of the library by voluntarily contributing to its support and extension. Such wide interest on the part of so many members of the Laboratory leading to numerous small gifts, whether in money or books, will be of the greatest value to the library. It is much appreciated! All the more because it is so broadly coöperative!

It is to be hoped that the Laboratory can soon supplement these gifts and place at the disposal of the library a sum to be expended in emergencies for the purchase of bargains which often become unexpectedly available. Such a fund of say $300 a year would make it possible to fill in gaps, complete sets, and add works which would be difficult to secure through more regular and slower channels. There is no more urgent need of the library than the maintenance of such an emergency fund, now so well started.

VI. THE DIRECTOR'S REPORT

To the Trustees of the Marine Biological Laboratory:

Gentlemen: I have the honor to present a report of the twenty-ninth session of the Marine Biological Laboratory for the year 1916. The total attendance was slightly smaller than in 1915, 231 as compared with 242, but larger than that of any earlier year. There were 129 investigators and 102 students, a falling off of 8 in the number of investigators and of 3 in the number of students. The decrease in investigators falls entirely under the head of investigation under instruction; indeed the number of independent investigators shows a gain of 5. The decrease should therefore be regarded as probably of a fluctuating character, as the group concerned consists largely of research students of members of the independent group of investigators. The slight decrease in student attendance was due entirely to our method of registration, for the number of applicants for courses was larger than ever before; after the courses were registered to the limit of our accommodations many students were refused registration. Later some of those registered failed to appear. It is obvious, therefore, that heads of departments should be allowed to register in excess of accommodations to a certain extent, in the expectation that a certain proportion of those registered will finally be unable to attend. The past season is the first one in which registration in advance was positively required, and this was done because the experience of 1915 had shown that unlimited registration was likely to exceed capacity, as indeed the number of applications in 1916 actually showed.

In spite of the slight decrease in numbers there was an increase in the receipts from investigators and students over all previous sessions, viz.: $8,725 in 1916 as compared with $8,325 in 1915, $7,300 in 1914, $6,160 in 1913, and $5,175 in 1912. The gain was entirely from the research side due to the fact that coöperating institutions subscribed $800 more in 1916 than in 1915 ($5,000 as against $4,200). The number of subscribing institutions was 40 in 1916 and 37 in 1915. The Supply Department has again shown a large increase of sales, which amounted to $21,096.65, as against $16,932.00 in 1915, $14,003.35 in 1914, $14,554.40 in 1913, $13,966.35 in 1912, $10,303.61 in 1911. The business has actually doubled in the last six years.

For several years the Director has called attention to need of increased accommodation for workers at the Laboratory in two directions: first the need of extra dormitory space for students, and second the need of cottages for the families of investigators, or of land on which such cottages could be placed. Both these needs now bid fair to be satisfied for some years to come. Mr. Crane has turned over to the Laboratory the Dexter House for use as a dormitory, with a sufficient sum of money to repair and equip it. We feel that with the other dormitory accommodations previously provided the students' needs will be adequately cared for. At the same time this extra accommodation enables us to dedicate the third floor of the Supply Department building, hitherto reserved for dormitory purposes, to the rapidly growing needs of this Department.

Provision has also been made for building sites for investigators by the purchase of 21 acres of woodland in the Gansett woods, immediately joining the Crow Hill region of Woods Hole, in which many of the Laboratory members now have homes, and extending from Quissett Ave. nearly to Buzzards Bay. This piece of land enjoys perpetual rights in a bathing reservation in a sandy cove of Buzzards Bay immediately adjoining. For convenience of location with reference to the Laboratory and to the sea no more desirable location could have been found; and there is no more beautiful tract in the entire town of Falmouth. Mr. Crane has furnished the money necessary to complete the purchase on favorable terms made by the owners, and he has also provided funds for its development, including the building of roads, which it is hoped may be accepted by the town. This work is now in progress, so that it is expected that parts of the land will be available for building purposes in the spring. A considerable number of requests for lots already have been received and some preliminary assignments made.

In view of the difficulty that has existed for so long in securing building sites for our membership and the probability that such difficulties are likely to increase in the future, the director recommends that none of this land be permanently alienated from the Laboratory by outright sale; and in order that it may be reserved for the purposes for which it was acquired he also recommends that none of it be otherwise disposed of except to members of the Laboratory unless by vote of the Board of Trustees in each case. Deeds of sale should, therefore, include a claim ensuring ultimate reversion to the Laboratory on equitable terms, and provisions should also be made for long term leaseholds. The form and value of the leasehold have occasioned much thought, and certain proposals are herewith presented for consideration by the Board. The valuations have been determined with no idea of speculative gain, but solely from the point of view of covering cost and expense of development with a small margin of safety for probable slowness of development. There is room obviously for difference of opinion with reference to values set on individual lots which have been determined as nearly as possible on the basis of general desirability. It will be found on comparison that the values thus determined contrast very favorably with the prices of building lots anywhere else in Woods Hole.

There is appended as part of this report a list of the staff, of investigators and students in attendance, a tabular view of attendance covering the last five years, a list of subscribing institutions, the Evening Lectures, and the Membership of the Corporation.

I. THE STAFF

1916

FRANK R. LILLIE, Director,
Professor of Embryology, and Chairman of the Department of Zoölogy,
The University of Chicago.

GILMAN A. DREW, Assistant Director, Marine Biological
Laboratory.

ZOÖLOGY

I. INVESTIGATION

Gary N. Calkins	Professor of Protozoölogy, Columbia University.

E. G. Conklin	Professor of Zoölogy, Princeton University.
Gilman A. Drew	Assistant Director, Marine Biological Laboratory.
George Lefevre	Professor of Zoölogy, The University of Missouri.
Frank R. Lillie	Professor of Embryology, The University of Chicago.
C. E. McClung	Professor of Zoölogy, University of Pennsylvania.
T. H. Morgan	Professor of Experimental Zoölogy, Columbia University.
E. B. Wilson	Professor of Zoölogy, Columbia University.

II. INSTRUCTION

Caswell Grave	Associate Professor of Zoölogy, Johns Hopkins University.
W. C. Allee	Professor of Biology, Lake Forest College.
George A. Baitsell	Instructor in Biology, Yale University.
Robert Hall Bowen	Graduate Student, Columbia University.
W. J. Kostir	Instructor in Zoölogy and Entomology, Ohio State University.
T. W. Painter	Adjunct Professor of Zoölogy, University of Texas.
Francis M. Root	Bruce Fellow, Johns Hopkins University.

EMBRYOLOGY

I. INVESTIGATION (See Zoölogy)

II. INSTRUCTION

William E. Kellicott	Professor of Biology, Goucher College.
Robert A. Budington	Professor of Zoölogy, Oberlin College.
Charles Packard	Instructor in Zoölogy, Columbia University.
Charles G. Rogers	Professor of Zoölogy, Oberlin College.
Reynold A. Spaeth	Instructor in Biology, Yale University.

PHYSIOLOGY

I. INVESTIGATION

Albert P. Mathews	Professor of Physiological Chemistry, The University of Chicago.
Ralph S. Lillie	Professor of Biology, Clark University.

Harold C. Bradley	Assistant Professor of Physiological Chemistry, University of Wisconsin.
Shiro Tashiro	Instructor in Physiological Chemistry, The University of Chicago.

II. INSTRUCTION

Ralph S. Lillie	Professor of Biology, Clark University.
Walter E. Garrey	Professor of Physiology, Tulane University.
Frank P. Knowlton	Professor of Physiology, Syracuse University.
A. R. Moore	Professor of Physiology, Rutgers College.

PHILOSOPHICAL ASPECTS OF BIOLOGY AND ALLIED SCIENCES

Lectures

Edward G. Spaulding	Professor of Philosophy, Princeton University.

BOTANY

George T. Moore	Director, Missouri Botanical Garden and Professor of Botany, Washington University.
Ivey F. Lewis	Professor of Biology, University of Virginia.
Carl S. Hoar	Instructor in charge of Botany, Williams College.

LIBRARY

H. E. McKnower	Professor of Anatomy, University of Cincinnati, Librarian.
Mary E. Scott	Assistant Librarian.

Chemical Supplies[1]

Oliver S. Strong	Instructor in Anatomy, College of Physicians and Surgeons, New York City, Chemist.

Supply Department

G. M. Gray	Curator.	A. M. Hilton	Collector.
John J. Veeder	Captain.	F. G. Gustafson	Collector in Botany.
E. M. Lewis	Engineer.		
A. W. Leathers	Collector.	Edna E. Wells	Clerk.

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[1] Compound microscopes, with two oculars and two objectives, rack and pinion and fine adjustment, may be rented for the seasons at $7.00 each, provided notice is received by the assistant director not later than June 15.

F. M. MacNaught	Business Manager.
Herbert A. Hilton	Superintendent of Buildings and Grounds.

2. INVESTIGATORS AND STUDENTS

1916

ZOÖLOGY

Independent Investigators

Addison, William H. F., Assistant Professor of Normal Histology and Embryology, University of Pennsylvania.
Allee, Warder C., Professor of Biology, Lake Forest College.
Allen, Ezra, Honorary Fellow, University of Pennsylvania.
Baitsell, George A., Instructor in Biology, Yale University.
Binford, Raymond, Professor of Zoölogy, Earlham College.
Bridges, Calvin B., Columbia University.
Budington, Robert A., Professor of Zoölogy, Oberlin College.
Calkins, Gary N., Professor of Protozoölogy, Columbia University.
Carothers, E. Eleanor, University of Pennsylvania.
Carver, Gail L., Professor of Biology, Mercer University.
Chidester, Floyd E., Associate Professor of Zoölogy, Rutgers College.
Child, Charles M., Associate Professor of Zoölogy, University of Chicago.
Clapp, Cornelia M., Professor Emeritus, Mount Holyoke College.
Conklin, E. G., Professor of Biology, Princeton University.
Copeland, Manton, Professor of Biology, Bowdoin College.
Cowdry, Edmund V., Associate in Anatomy, Johns Hopkins Medical School.
Cowdry, N. H., Johns Hopkins Medical School.
Crampton, H. E., Professor of Zoölogy, Barnard College, Columbia University.
Donaldson, Henry H., Professor of Neurology, Wistar Institute.
Drew, Gilman A., Assistant Director, Marine Biological Laboratory, Woods Hole.
Gee, Wilson, Professor of Biology, Emory University.
Goldfarb, A. J., Assistant Professor of Biology, College of the City of New York.
Goldschmidt, Richard, Kaiser Wilhelm Institut für Biologie.
Goodrich, Hubert B., Instructor in Biology, Union College.
Grave, Caswell, Associate Professor of Zoölogy, Johns Hopkins University.
Gregory, Emily R., University of Pennsylvania.
Gross, Alfred O., Assistant Professor of Zoölogy, Bowdoin College.
Guyer, Michael F., Professor of Zoölogy, University of Wisconsin.
Heilbrunn, Lewis V., Associate in Embryology, University of Chicago.
Hogue, Mary J., Instructor in Zoölogy, Wellesley College.
Hoskins, Elmer R., Instructor in Anatomy, New York University.
Jacobs, Merkel H., Assistant Professor of Zoölogy, University of Pennsylvania.
Kellicott, William E., Professor of Biology, Goucher College.
Kernall, Morris J., University of Illinois.
Knower, Henry McE., Professor of Anatomy, University of Cincinnati.
Lefevre, George, Professor of Zoölogy, University of Missouri.
Lillie, Frank R., Professor of Embryology, University of Chicago.
Lund, Elmer J., Assistant Professor of Zoölogy, University of Minnesota.

McClung, Clarence E., Director of Zoölogy Laboratories, University of Pennsylvania.
Malone, Edward F., Associate Professor of Anatomy, University of Cincinnati.
Moodie, Roy L., Associate in Anatomy, University of Illinois.
Moore, Carl R., Assistant in Embryology, University of Chicago.
Morgan, Thomas H., Professor of Experimental Zoölogy, Columbia University.
Morris, Margaret, Yale University.
Muller, Hermann J., Instructor, Rice Institute.
Newman, Horatio H., Associate Professor of Zoölogy, University of Chicago.
Packard, Charles, Instructor in Zoölogy, Columbia University.
Pappenheimer, Alvin M., Assistant Professor of Pathology, Columbia University.
Patten, William, Professor of Biology, Dartmouth College.
Peebles, Florence, Professor of Biology, H. Sophie Newcomb Memorial College.
Reagen, F. P., Princeton University.
Richards, A., Adjunct Professor of Zoölogy, University of Texas.
Rogers, Charles G., Professor of Comparative Physiology, Oberlin College.
Shull, A. Franklin, Associate Professor of Zoölogy, University of Michigan.
Shumway, Waldo, Instructor in Biology, Amherst College.
Spaeth, Reynold A., Instructor in Biology, Yale University.
Spaulding, Edward G., Professor of Philosophy, Princeton University.
Stockard, Charles R., Professor of Anatomy, Cornell Medical College.
Strong, Oliver S., Instructor in Anatomy, Columbia University.
Strong, Archibald M., Instructor in Medicine, Columbia University.
Sturtevant, Alfred H., Columbia University.
Taylor, Herbert D., Assistant in Pathology and Bacteriology, Rockefeller Institute.
Tennent, David H., Professor of Biology, Bryn Mawr College.
Wenrich, David H., Instructor in Zoölogy, University of Pennsylvania.
Weinstein, Alexander, Fellow, Columbia University.
Welch, Paul S., Associate Professor of Entomology, Kansas State Agricultural College.
Whiting, Phineas W., Research Fellow, University of Pennsylvania.
Wieman, Harry L., Head of Dept. of Zoölogy, University of Cincinnati.
Wilson, E. B., Professor of Zoölogy, Columbia University.
Woodward, Alvalyn E., Instructor, Vassar College.

Beginning Investigators

Blake, Irving H., Dept. of Zoölogy and Physiology, Oregon Agricultural College.
Brumfiel, Daniel M., Instructor in Animal Biology, University of Iowa.
Chapin, Catharine L., University of Chicago.
Cohn, Edwin J., University of Chicago.
Davies, I. J., Micrologist, Rice Institute.
Globus, Joseph H., Assistant in Anatomy, Cornell Medical School.
Goldman, Agnes, Cornell Medical School.
Heilbrunn, Stella, Teacher, Bay Ridge High School, Brooklyn.
Herman, William, Harvard Medical School.
Hoy, William E., Jr., Instructor, University of Rochester.
Huxley, Julian S., Assistant Professor of Biology, Rice Institute.
Lynch, Clara J., 925 W. Cleveland Ave., Canton, Ohio.

MacArthur, John W., Associate in Zoölogy, University of Chicago.
MacMorland, Edward E., Fellow in Biology, Princeton University.
Cooper, Arthur R., Fellow in Zoölogy, University of Illinois.
Minoura, Tadachika, University of Chicago.
Montgomery, Mrs. Priscilla B., University of Pennsylvania.
Parmenter, Charles L., Assistant, University of Minnesota.
Pinney, Mary E., Demonstrator in Biology, Bryn Mawr College.
Root, Francis M., Fellow, Johns Hopkins University.
Safir, Shelley R., Teacher in Stuyvesant High School, New York City.
Smith, Christianna, Assistant, Zoölogy Department, Mt. Holyoke College.
Speidel, Carl C., Graduate Student, Princeton University.
Ware, Clara C., Assistant at Barnard College, Columbia University.
White, Edith G., Research Assistant, Princeton University.

PHYSIOLOGY

Independent Investigators

Chambers, Robert, Jr., Instructor in Anatomy, Cornell Medical College.
Day, Edward C., Lecturer in Physiology, Bryn Mawr College.
Duesberg, Jules, Research Associate, Carnegie Institution.
Edwards, Dayton J., Instructor in Physiology, College of City of New York.
Ellis, Frederick W., Monson, Mass.
Garrey, Walter E., Professor of Physiology, Tulane University.
Harvey, E. Newton, Assistant Professor of Physiology, Princeton Univ.
Harvey, Mrs. E. Newton, Princeton, N. J.
Knowlton, Frank P., Professor of Physiology, Syracuse University.
Kudo, R., Rockefeller Institute for Medical Research.
Lillie, Ralph S., Professor of Biology, Clark University.
Loeb, Jacques, Head of Department of Experimental Biology, Rockefeller Institute for Medical Research.
Lyon, Elias P., Professor of Physiology, University of Minnesota.
Mathews, Albert P., Professor of Physiological Chemistry, University of Chicago.
Moore, Arthur R., Professor of Physiology, Rutgers College.
Noguchi, Hideyo, Research Member, Rockefeller Institute for Medical Research.
Northrop, John H., Assistant, Rockefeller Institute for Medical Research.
Patten, Bradley M., Senior Instructor, Western Reserve Medical School.
Price, Weston A., Chairman, Research Commission of the National Dental Association, Cleveland, Ohio.
Richards, Annabella E., Demonstrator of Chemistry, Woman's Medical College of Pennsylvania.
Rudisch, Julius, Consulting Physician, Mount Sinai Hospital, New York.
Van der Stricht, O., Western Reserve Medical School.
Wasteneys, Hardolph, Associate in Experimental Biology, Rockefeller Institute for Medical Research.

Beginning Investigators

Adler, Francis H., University of Pennsylvania.
Chamberlain, Mary M., Fellow, University of Pennsylvania.
Noble, Arlyle, Assistant in Bacteriological Research, Parke Davis & Co.

BOTANY

Independent Investigators

Lewis, Ivey F., Professor of Biology, University of Virginia.
Lyman, George R., Pathological Inspector, Dept. of Agriculture, Washington, D. C.
Moore, George T., Director, Missouri Botanical Garden, St. Louis, Mo.
Moore, Emmeline, Instructor, Vassar College.
Osterhout, W. J. V., Professor of Botany, Harvard University.
Snow, Laetitia M., Associate Professor of Botany, Wellesley College.
Weston, William H., Jr., Instructor in Biology, Adelbert College.

Beginning Investigator

Haas, Albert R., Graduate Student, Harvard University.

STUDENTS

1916

BOTANY

Browning, Harold W., University of Wisconsin, Madison, Wis.
Clum, Harold H., Student, Oberlin College, Oberlin, Ohio.
Donovan, Stephen M., Student, St. Bonaventure, St. Bonaventure, N. Y.
de Angulo, Mrs. Cary F., Carmel, California.
Furber, Jane M., 769 Washington St., Brookline, Mass.
Jones, Winifred, Teacher, Cincinnati Public Schools, Cincinnati, Ohio.
McNair, Frances D., Smith College, Northampton, Mass.
McWhorter, Frank P., Vanderbilt University, Nashville, Tenn.
Martin, George W., Instructor, Mass. Agricultural College, Amherst, Mass.
Page, Frederick S., Instructor, Dartmouth College, Hanover, N. H.
Reeves, Walter S., Missouri Botanical Garden, St. Louis, Mo.
Smith, Edna L., Carmel, California.

EMBRYOLOGY

Baker, Ethel C., 123 Chiswick Road, Boston, Mass.
Beyer, Henry G., U. S. Navy, Retired.
Bodine, Joseph H., Assistant in Zoölogy, University of Pennsylvania.
Bowen, Robert H., 330 Summit Ave., Mt. Vernon, N. Y.
Charlton, Harry H., North Billerica, Mass.
Druien, Mildred M., Vassar College, Poughkeepsie, N. Y.
Hague, Florence, Assistant, Kansas State Agricultural College.
Hoagland, Ruth A., Assistant in Zoölogy, Vassar College.
Hopkins, Hoyt S., Johns Hopkins University, Baltimore, Md.
Job, Thesle T., Fellow, State University of Iowa.
Lopez-Suarez, Juan, Madrid.
McRoberts, Earl S., Butler College, Indianapolis, Ind.
Mahr, Ernest F., Syracuse University, Syracuse, N. Y.
Mason, Michael L., Northwestern University.
Moore, Dorothea M., Radcliffe College, Cambridge, Mass.

Myers, Dorothy K., Barnard College, New York City.
Parker, Sylvia L., Laboratory Assistant, Mt. Holyoke College.
Rae, Anne M., Teacher, Girls High School, Brooklyn, N. Y.
Ripple, C. Vincent, Instructor, South Philadelphia High School.
Rowland, Edith L., Barnard College, New York City.
Schneider, Peter A., Instructor, University of Vermont.
Shaw, Myron E., Student, Carleton College.
Smith, Elizabeth A., Instructor in Zoölogy, University of Wisconsin.
Sprowles, Edith A., Teacher, Frankford High School, Frankford, Pa.
Vaupel, Jean, University of Cincinnati.
Wardwell, Edward H., Graduate Student, Brown University.

PHYSIOLOGY

Chase, Samuel W., Student, Harvard University.
Childs, Henry E., Teacher, Rochester, N. Y.
Greisheimer, Esther, Clark University.
Hunt, Harrison R., Student, Harvard University.
Irwin, Marion, 53 Garden St., Cambridge, Mass.
Millikin, Frances, Smith College, Northampton, Mass.
Mudd, Stuart, Princeton, N. J.
Mühl, Anita M., Butler College, Indianapolis, Ind.
Pond, Samuel E., Fellow, Clark University.
Runyon, Paul M., Student, Princeton University.
Sampson, Myra M., Instructor, Smith College.
Twining, Ralph H., Clark University.
Young, Paul T., Graduate Student, Princeton University.
Young, Eldrid G., 4876 Sherbrooke St., Montreal.

ZOÖLOGY

Bach, Ellen B., 721 S. Ingalls St., Ann Arbor, Mich.
Bargen, Jacob A., Student, Carleton College.
Bickerstaff, Florence W., Student, Oberlin College.
Biddle, M. Georgina, 2017 De Lancey Place, Philadelphia, Pa.
Blatchford, Barbara, Student, Vassar College.
Brenton, Thaddeus R., Laboratory Assistant in Zoölogy, Moores Hill College.
Brumback, Florence M., Teacher, Waukegan Township High School.
Brunswick, Mark, Quissett, Mass.
Bryant, Gladys, Student, Radcliffe College.
Choate, Mabel, Barnard College, Columbia University.
Cauldwell, Olivia, Student, Vassar College.
Colvill, Helen, Teacher, Circleville, Ohio.
Davis, Gordon E., Oberlin College.
Dawson, James A., Assistant in Biology, Yale University.
Detwiler, Samuel R., Graduate Student, Yale University.
de la Cruz, Dionysia G., Student, Sophie Newcomb College.
Ellinger, Margaret, 14 W. 25th St., Baltimore, Md.
Felty, Augustus R., 734 Prospect Ave., Hartford, Conn.
Herrick, Ruth, University of Chicago.
Hinrichs, Marie A., 1832 Blue Island Ave., Chicago, Ill.

Houlding, Ernest W., 182 E. College St., Oberlin, Ohio.
Lamb, Laura, 527 S. 41st St., Philadelphia, Pa.
Levien, Eunice M., Lake Forest College.
Leigh, Howard, Earlham College,
Mack, Ruth J., Radcliffe College, Cambridge, Mass.
Neun, Elsie G., University of Rochester.
Nute, Bertha E., Mount Holyoke College.
Roach, Ethel S., University of Rochester.
Rorer, Mary D., Student, Mount Holyoke College.
Rowe, Georgia M., 2321 N. Calvert St., Baltimore, Md.
Ruhl, Rebecca L., 205 E. Main St., Clarksburg, W. Va.
Seago, Mary M., Student, Sophie Newcomb College.
Skillern, Inez, Sweet Briar College, Sweet Briar, Va.
Stewart, Eleanor G., 425 St. Clair St., Pittsburgh, Pa.
Stewart, Fred W., Schuyler Fellow, Cornell University.
Swann, Mrs. Arthur W., 124 East 61st St., New York City.
Taylor, Martha, Student, Radcliffe College.
Tilghman, Sarah A., Goucher College.
Turk, Irene, Hunter College, New York City.
VerNooy, Lester C., Amherst College.
Wakefield, Lucile, Student, Carnegie Institute of Technology.
Walker, Louise, Laboratory Assistant, Brearley School, New York City.
Walters, Mary J., Goucher College, Baltimore, Md.
Walton, Everett P., Topsham, Maine.
Wasserman, Jeanette H., Hunter College, New York City.
White, Gertrude M., Assistant, University of Wisconsin.
Weinstein, Florence, Student, Barnard College.
Welch, M. W., 1806 Morse Ave., Chicago, Ill.
Wenrich, Myra S., Philadelphia, Pa.
Yagle, Elizabeth M., Student, Carnegie Institute of Technology.

3. TABULAR VIEW OF ATTENDANCE

	1912	1913	1914	1915	1916
Investigators—Total	93	122	129	137	129
Independent:					
Zoölogy	44	58	62	69	70
Physiology	14	17	22	20	23
Botany	10	11	10	6	7
Under Instruction:					
Zoölogy	21	21	31	36	25
Physiology	2	7	1	4	3
Botany	2	7	3	2	1
Students—Total	67	69	89	105	102
Zoölogy	24	33	43	47	50

Embryology	15	22	21	37	26
Physiology	11	8	10	15	14
Botany	17	7	15	6	12
TOTAL ATTENDANCE	160	191	218	242	231
INSTITUTIONS REPRESENTED—Total	57	80	77	79	73
By investigators	43	50	51	59	51
By students	36	41	47	42	45
SCHOOLS AND ACADEMIES REPRESENTED:					
By investigators	2	3	1	3	—
By students	1	6	5	9	3

4. SUBSCRIBING INSTITUTIONS—1916

AMHERST COLLEGE
BARNARD COLLEGE
BOWDOIN COLLEGE
BRYN MAWR COLLEGE
CARLETON COLLEGE
CARNEGIE INSTITUTE OF TECHNOLOGY
CLARK UNIVERSITY
COLUMBIA UNIVERSITY
DARTMOUTH COLLEGE
ELSE SERINGHAUS SCHOLARSHIP, HUNTER COLLEGE, NEW YORK CITY
GOUCHER COLLEGE
HARVARD UNIVERSITY
JOHNS HOPKINS UNIVERSITY
KANSAS STATE AGRICULTURAL COLLEGE
KAISER WILHELM INSTITUT FÜR BIOLOGIE
LAKE FOREST COLLEGE
MOUNT HOLYOKE COLLEGE
NORTHWESTERN UNIVERSITY
OBERLIN COLLEGE
PRINCETON UNIVERSITY, DEPT. OF BIOLOGY
PRINCETON UNIVERSITY, DEPT. OF PSYCHOLOGY
RADCLIFFE COLLEGE
ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
RICE INSTITUTE
RUTGERS COLLEGE
SMITH COLLEGE

H. Sophie Newcomb Memorial College
University of Chicago
University of Cincinnati
University of Illinois
University of Kansas
University of Michigan
University of Pennsylvania
University of Rochester
University of Texas
University of Wisconsin
Vassar College
Wellesley College
Wistar Institute
Yale University

5. EVENING LECTURES, 1916

Friday, June 30,	Prof. W. J. V. Osterhout	"Permeability and Antagonism."
Friday, July 7,	Prof. Jacques Loeb	"On Adaptation to Environment."
Tuesday, July 11,	Dr. Robert M. Yerkes	"The Study of Monkeys and Apes."
Friday, July 14,	Dr. James B. Murphy	"Studies in Lymphoid Activity."
Tuesday, July 18,	Prof. Alexander Petrunkevitch	"Segmentation in Arthropods."
Friday, July 21,	Prof. J. Lawrence Henderson	"Organization as a Physical Chemical Problem."
Tuesday, July 25,	Prof. W. B. Cannon	"Some Recent Studies of Glands of Internal Secretion."
Friday, July 28,	Prof. Richard Goldschmidt	"Experimental Intersexuality and the Sex-problem."
Tuesday, Aug. 1,	Dr. Raymond Pearl	"Parental Alcoholism and the Progeny in Poultry."

Tuesday, Aug. 8,
Dr. Alfred G. Mayer . . . . "Biological Problems of the Pacific."

MEMBERS OF THE CORPORATION

Life Members

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Md.
Billings, Mr. R. C., 66 Franklin St., Boston, Mass.
Carey, Mr. Arthur Astor, Fayerweather St., Boston, Mass.
Clarke, Prof. S. F., Williamstown, Mass.
Conklin, Prof. Edwin G., Princeton University, Princeton, N. J.
Crane, Mr. C. R., Woods Hole, Mass.
Davis, Major Henry M., Syracuse, N. Y.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Mass.
Farlow, Prof. W. G., Harvard University, Cambridge, Mass.
Fay, Miss S. B., 88 Mt. Vernon St., Boston, Mass.
Folsom, Miss Amy, 88 Marlboro St., Boston, Mass.
Foot, Miss Katherine, 955 Park Ave., New York City, N. Y.
Gardiner, Mrs. E. G., Woods Hole, Mass.
Gardiner, Miss Eugenia, 15 W. Cedar St., Boston, Mass.
Harrison, Ex-Provost C. C., University of Pennsylvania, Philadelphia, Pa.
Jackson, Miss M. C., 88 Marlboro St., Boston, Mass.
Jackson, Mr. Chas. C., 24 Congress St., Boston, Mass.
Kennedy, Mr. Geo. G., 284 Warren St., Roxbury, Mass.
Kidder, Mr. C. G., 27 William St., New York City, N. Y.
Kidder, Mr. Nathaniel T., Milton, Mass.
King, Mr. Chas. A.
Lee, Mrs. Frederic S., 279 Madison Ave., New York City, N. Y.
Lowell, Mr. A. Lawrence, 17 Quincy St., Cambridge, Mass.
Marrs, Mrs. Laura Norcross, 9 Commonwealth Ave., Boston, Mass.
Mason, Miss E. F., 1 Walnut St., Boston, Mass.
Mason, Miss Ida M., 1 Walnut St., Boston, Mass.

Means, Mr. James Howard, 196 Beacon St., Boston, Mass.
Merriman, Mrs. Daniel, 73 Bay State Road, Boston, Mass.
Minns, Miss Susan, 14 Louisburg Square, Boston, Mass.
Minns, Mr. Thomas, 14 Louisburg Square, Boston, Mass.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Sts., New York City, N. Y.
Morgan, Prof. T. H., Columbia University, New York City, N. Y.
Morgan, Mrs. T. H., New York City, N. Y.
Noyes, Miss Eva J.
Nunn, Mr. Lucian L., Telluride, Colo.
Osborn, Prof. Henry F., American Museum of Natural History, New York City, N. Y.
Phillips, Dr. John C., Windy Knob, Wenham, Mass.
Phillips, Mrs. John C., Windy Knob, Wenham, Mass.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pa.
Pulsifer, Mr. W. H., Newton Center, Mass.
Rogers, Miss A. P., 5 Joy St., Boston, Mass.
Sears, Dr. Henry F., 86 Beacon St., Boston, Mass.
Shedd, Mr. E. A.
Smith, Mrs. C. C., 286 Marlboro St., Boston, Mass.
Strobell, Miss E. C., 955 Park Ave., New York City, N. Y.
Thorndike, Dr. Edward L., Teachers College, Columbia University, New York City, N. Y.
Trelease, Prof. William, University of Illinois, Champaign, Ill.
Ware, Miss Mary L., 41 Brimmer St., Boston, Mass.
Whitney, Mr. Henry M., Brookline, Mass.
Wilcox, Miss Mary A., Wellesley College, Wellesley, Mass.
Wilmarth, Mrs. H. D., Elliott St., Jamaica Plain, Mass.
Williams, Mrs. Anna P., 505 Beacon St., Boston, Mass.
Wilson, Dr. E. B., Columbia University, New York City, N. Y.
Wilson, Prof. W. P., Commercial Museum, Philadelphia, Pa.

7. CORPORATION MEMBERSHIP LIST

January, 1917.

Abbott, Prof. J. F., Washington University, St. Louis, Mo.
Abbott, Miss Margaret B., The Bennett School, Milbrook, N. Y.

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STUDIES ON THE BIOLOGY OF PARACOPIDOSOMOPSIS
II. SPERMATOGENESIS OF MALES REARED FROM UNFERTILIZED EGGS.[1]

J. T. PATTERSON AND LELIA T. PORTER.

In the first paper of this series it was pointed out that the unfertilized egg of *Paracopidosomopsis floridanus* produces a polyembryonic brood of males. It is the purpose of this paper to show that the germ cells of such males are characterized by the haploid number of chromosomes. As in many other Hymenoptera, the presence of the half number is due to the fact that in the parthenogenetic development of the matured egg there is no compensatory process for restoring the full number. As a consequence of the reduced number of chromosomes in these males, the maturation divisions are modified in such a way that two, instead of four, spermatozoa are produced from each first spermatocyte. In the somatic cells of these males the haploid number, subject to certain variations, also prevails; but this is a subject that will be considered later.

Material and Methods.

All of the material used in the study of spermatogenesis has been taken from animals reared under experimental conditions. It consists of two large broods of larvæ and a series of one hundred pupæ taken from a mummified carcass of the host. The two larval broods were preserved forty-eight and twenty-four hours, respectively, prior to the time at which pupation would have occurred. The series of pupa stages were preserved at close intervals, beginning twenty-four hours after the formation of the carcass.

The three broods yielding this material resulted from an experiment designed to determine whether the unfertilized egg developed into a brood of males. A summary of the experiment is as follows:

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[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 131.

Host eggs laid the night of November 7.
Each egg parasitized by one oviposition of an unfertilized female, 2:30 P.M., Nov. 8.
Caterpillars hatched Nov. 13.
First brood of parasites preserved Dec. 4.
Second brood of parasites preserved Dec. 5.
Carcass of third caterpillar formed Dec. 6.
Series of pupa stages from carcass preserved Dec. 7–19.
Brood of 1842 male parasites emerged Dec. 25.

We have used a great variety of fixing fluids on polyembryonic material, but for the cytology of male germ cells no reagent has been found that equals Bouin's picro-formol-acetic mixture to which crystals of urea have been added. Preparations of the gonads fixed in this fluid and treated with Heidenhain's iron hæmatoxylin, counterstained with Orange G, give very fine results. However, this method of fixation has the disadvantage of not being adapted to the staining technique usually employed to bring out certain cytoplasmic structures, such as the mitochondria. Nevertheless, we have used it almost exclusively because of our especial interest in the chromosomes.

Even with the use of the best methods of technique, Hymenoptera material is never as favorable for the study of chromosomes as that of many other insects, notably the Hemiptera and Orthoptera. In certain respects, the germ cells of *Paracopidosomopsis* are more favorable for cytological study than those of many other species of the order, but even in this species the chromosomes are, at certain stages, greatly elongated, with a consequent tendency to become entangled with one another. This often makes difficult the determination of the exact number. The difficulty is somewhat increased by the smallness of the cell, which at the end of the growth period does not exceed a diameter of seven or eight microns. The best counts of chromosome numbers are obtained in somatic cells, because in these cells the metaphase plate is flat and the chromosomes are more scattered than in the spermatocytes. In any event, one does not experience difficulty in distinguishing a metaphase plate with the haploid number of chromosomes from one having the diploid number.

The Spermatogonia.

We have not attempted to follow closely the course of development of the male gonads in the larva. At the earliest stage at which the larva can be recognized as a male, each testis consists of a solid spherical mass of cells. Histologically, there are two kinds of cellular elements, germ cells and epithelial cells. During the course of further development the gonad elongates, and at the same time cyst formation occurs. This process is completed by the end of the larval period.

At no time during the development of the gonad does one find any great number of spermatogonial divisions. Four or five mitotic figures are the most that will be found at any one time in a given testis.

The period of multiplication ends sometime between forty-eight and twenty-four hours prior to pupation. Of the two larval broods mentioned above, the one preserved forty-eight hours before the time of pupation has a few larvæ showing spermatogonial divisions, while the one fixed twenty-four hours later has no divisions.

Fig. 1 shows a metaphase plate of one of the dividing spermatogonia. The plate has eight elongated chromosomes, one of which shows the beginning of a longitudinal split. We have concluded from a study of all of our material of both males and females that eight represents the haploid number of chromosomes.

The Growth Period.

The growth period begins shortly after the cessation of the spermatogonial divisions, and extends through the fourth day after pupation. At the beginning of this period all of the germ cells in a given cyst are in the same stage of development. In section each cell appears wedge-shaped, with its slightly curved base lying against the outer membrane and its apex directed toward the center of the cyst. The apexes of all the cells in the cyst are usually connected by a common mass, which is apparently the remains of the interzonal fibers of the last spermatogonial division.

The growth stage is characterized by the presence of a conspicuous nucleolus, which occupies an excentric position in the nucleus (Figs. 2–4). At first the nucleolus is somewhat diffuse, but later its outline becomes sharp and distinct (Fig. 2). It frequently has several less deeply stained areas lying at its center (Fig. 3). We have good reasons for believing that the nucleolus is chromatin in character.

One of the most striking changes to occur in the cell during growth, is the appearance of certain cytoplasmic inclusions, which stain intensely black with iron hæmatoxylin. These are first seen about seventy-two hours after pupation, when they appear as dark, elongated areas in the cytoplasm. Later, as the cell enlarges, the areas become more definite and appear as seen in Fig. 3. These structures soon disappear (Figs. 4, 5), although some of them may persist until after the first maturation division is well advanced (Figs. 6, 8). We have not attempted to determine the origin and nature of these cytoplasmic inclusions; but, judging from the work of Meves on the bee, they are probably mitochondrial in character.

The presence of these inclusions greatly interferes with the study of the changes which now take place in the cell. Especially is this true with reference to those changes involving the behavior of the centrosomes. We shall therefore confine our account to the changes in shape of the cell which occur during the growth period. As already stated, all of the cells of a cyst are at first united at their pointed ends by the interzonal connections. In some cells these connections are soon lost, and each cell then becomes polyhedral in outline (Figs. 2, 3), and finally spherical. In other cells the interzonal connections apparently persist throughout the entire growth period, and after these are finally severed, the pointed end of each primary spermatocyte contains the remains of the original interzonal connection in the form of an "interzonal body" (Mark and Copeland).

At the end of the growth period many cells are met with which possess a delicate process. It is difficult to demonstrate the presence of a centrosome at the end of this process (Fig. 4). With reference to the position of the cell in the cyst, the process may lie on any side of the cell. It is not clear whether such a process is formed in every cell. If it is of constant occurrence, the period of its existence must be very brief; for at the beginning of the first spermatocyte division, which soon follows, it is entirely absent.

A similar process has been described for the young spermatocytes of several different Hymenoptera, but the most careful and detailed work on it is that of Meves ('07) on the honey bee. According to Meves, the process in the bee is formed through the influence of one of the primary centrosomes ("Hauptcentriolen"), which arise by division from the original centrosome of the cell. The process with its centrosome is soon withdrawn into the cell. One of the several secondary centrosomes ("Nebencentriolen") which had previously arisen by division from the primary centrosomes, then participates in the formation of the mitotic figure responsible for the production of the so-called polar body.

While it is not possible in our material to demonstrate all of these fine points, yet it is highly probable that the cytoplasmic process in the germ cells of *Paracopidosomopsis* has a similar history to that described by Meves for the germ cells of the bee.

First Maturation Division.

Preparation for the first maturation division is made manifest by changes in the nucleus. It is also made evident by changes in the shape of the cell, especially in the spermatocytes which have early lost their interzonal connections. In cells in which these connections persist up until the end of the growth period, the so-called polar body, or rudimentary second spermatocyte, is budded off from the side of the cell that had recently had the connection. In the other spermatocytes a blunt protrusion arises at one side (Fig. 5), which soon develops into a pointed process (Fig. 6). At the pointed end thus formed there is present a tiny centrosome, from which radiate a number of delicate fibers toward the nucleus. At the opposite, or blunt end of the cell, a similar centrosome with its fibers is also present.

The most important change in the nucleus concerns the nucleolus, which during the first half of the growth period remains a deeply staining, spherical mass (Figs. 2, 3). A number of spherical granules arise at its periphery, giving it the appearance of a lobulated structure (Fig. 4). Eventually the nucleolus breaks up into a number of these large granules, which lie in a finely granular matrix (Fig. 5). Apparently these larger granules coalesce to form the chromosomes (Fig. 7). From a careful study of this particular stage, one can not escape the conclusion that the chromosomes arise from the nucleolus.

A somewhat similar method of origin of chromosomes has been described by Meves and Duesberg ('08) in the male germ cells of *Vespa crabro*. They say: "Das Herannahen der ersten Reifungsteilung macht sich dadurch bemerkbar, das im Kern in der Umgebung des grösseren Nucleolus immer mehr Chromatinkörner auftreten. Der Nukleolus selbst wird dabei immer kleiner. Man gewinnt den Eindruck, dass seine Substanz in diejenige der Chromatinkörner übergeht." There is one important difference between the two forms. In *Paracopidosomopsis* the nucleolus gradually but completely disappears as the chromosomes are formed, while in *Vespa* a small body still remains after the chromosomes are organized.

During the organization of the chromosomes the nucleus elongates in the direction of the long axis of the cell. An imperfect intranuclear spindle then arises, and upon this the chromosomes tend to take up an equatorial position (Figs. 6, 8). They are usually so closely massed together that it is impossible to make an exact determination of their number. Fig. 6 shows a very interesting case, in which a single curved chromosome has moved to the upper end of the nucleus, where it lies in contact with the inner surface of the nuclear membrane. On account of the massed condition of the chromosomes, it is not possible to tell whether this particular chromosome is the product of a recent division, and has a sister chromosome lying within the mass. However, this is an isolated case, and it is clear that the chromosomes do not normally divide in the first spermatocyte division. Instead, only the cytoplasm undergoes division, and this in a very adequal manner (Figs. 7, 8).

The constriction of the cytoplasm begins about one-third the distance from the pointed end of the cell (Fig. 7) and finally results in cutting off a small, knob-like mass entirely free of chromatin (Fig. 9). This abortive division is homologous to the first spermatocyte division which normally occurs in other forms, and the two unequal cells thus produced are to be regarded as equivalent to second spermatocytes.

The small non-nucleated cell, or mass of cytoplasm, soon degenerates, although in some cases it may persist until the second maturation division is well advanced. All stages in the disintegration of the mass are to be seen in a single cyst. The fragmenting masses lie in the interstices of the dividing second spermatocytes.

The most interesting point to be noted in connection with this abortive maturation division is the fact that nothing comparable to a synapsis of chromosomes occurs. The absence of this phenomenon, which is universal in the spermatogenesis of males arising from fertilized eggs, is undoubtedly due to the fact that the unmatured, male germ cell of *Paracopidosomopsis* possesses but the haploid number of chromosomes. The function of the first maturation division is to bring about a reduction from the diploid to the haploid number of chromosomes; but the males of this species, by virtue of their origin from matured, but unfertilized eggs, already possess the half number, and consequently, the first maturation results in a feeble or abortive attempt at a division.

Second Maturation Division.

Throughout the entire period occupied by the first maturation, the nuclear membrane remains intact (Figs. 5–9). At the end of this period the incomplete intranuclear spindle disappears, as do also the fibers which radiate from the centrosome included in the small cytoplasmic mass (Fig. 9). On the contrary, the aster, which lies at the lower side of the nucleus in the true second spermatocyte, persists. It is highly probable that this aster, by a division of its centrosome, gives rise to the second maturation spindle, as is the case in the European hornet.

In the brief interim between the first and second maturations, the nucleus enters a rest stage. The chromatin recedes to that side of the nucleus lying farthest from the cytoplasmic bud, and becomes so massed that it is impossible longer to distinguish clearly individual chromosomes (Fig. 9). In the stages immediately following this one, every phase in the reorganization of the chromosomes is clearly demonstrable. In having a definite resting stage between the abortive and the true division, *Paracopidosomopsis* resembles *Neuroterus* (Doncaster, '09), but differs from such forms as the bee and the wasp.

After the cytoplasmic bud is cut off, the second spermatocyte becomes spherical in outline, and a conspicuous maturation spindle is formed (Fig. 12). The reorganized chromosomes are drawn into the equatorial position on this spindle. Clear polar views are difficult to find, owing to the fact that the chromosomes are elongated and frequently twisted (Fig. 12). In the clearest cases observed, the number of chromosomes is seen to be eight (Figs. 10, 11).

The axis of the second maturation spindle bears no definite relation to that of the first maturation spindle. This was determined by studying those second spermatocytes to which the cytoplasmic bud remains for some time slightly attached. It was found that the axis of the second spindle has no definite relation to the point of attachment.

In the second division the chromosomes split lengthwise, and the daughter chromosomes pass to the opposite poles of the spindle (Fig. 14). It is frequently easy to count the eight daughter chromosomes passing to the one or the other of the poles, but cells in which both daughter groups can be counted are not often met with (Fig. 15). We conclude from these observations that all of the chromosomes divide in the second maturation. While in the anaphases the chromosomes are often well scattered, yet we have found no evidence of a distinctly "advancing" or "lagging" member, such as might indicate that a particular chromosome had failed to divide. From this it follows that the two spermatids resulting from the second division will be alike. That is to say, the spermatozoa in *Paracopidosomopsis* will not be dimorphic.

In passing to the poles of the spindle the chromosomes keep their long axes parallel to the long axis of the spindle (Figs. 15, 16), and upon reaching the pole fuse to form a solid, deeply staining mass of chromatin out of which the spermatid nucleus arises (Figs. 17, 18).

After the division of the cytoplasm, the two spermatids remain connected for some time by means of the interzonal fibers (Fig. 18). After they separate, each differentiates into a typical spermatozoön (Figs. 19, 20). We shall not discuss further the subject of the metamorphosis of the spermatids, except to emphasize the point that both spermatids must form functional germ cells, as we have found no evidence of degenerating spermatids or spermatozoa.

COMPARISON WITH OTHER HYMENOPTERA.

Studies on the spermatogenesis of Hymenoptera have shown that there is a striking modification of the maturation process in those species in which sex-determination is supposed to be in accordance with the Dzierzon theory. Instead of four spermatozoa arising from each first spermatocyte, only one or two matured germ cells develop. In one group of these Hymenoptera both spermatocyte divisions are abortive; the first division cutting off a small cytoplasmic bud free of chromatin, and the second division a small bud which receives half the chromatin. There is thus produced but a single spermatid, and consequently only one spermatozoön. In the second group the first division is likewise abortive, producing a cytoplasmic bud, but the second division is equal, producing two similar spermatids, which metamorphose into spermatozoa.

To the first of these groups belongs the honey bee, as has been shown by the work of Meves ('03, '07), Mark and Copeland ('06), and Doncaster ('06, '07). To this class there also probably belongs the solitary bee (*Osmia cornuta*) worked on by Armbruster ('13).

The second group includes a number of different species, of which the following may be mentioned: *Xylocopa violacea* (Granata, '10, '13), *Neuroterus lenticularis* (Doncaster, '09), *Vespa crabro* (Meves and Duesberg, '08), *Vespa maculata* (Mark and Copeland, '07), *Vespa germanica* (Meves, '03), *Camponotus herculaneus* (Meves and Duesberg, '08, and Lams, '08), *Dryophanta erinacei* (Wieman, '15). *Paracopidosomopsis floridanus* also clearly belongs to this class.

While the various investigators report differences as to details in the two types of spermatogenesis outlined above, yet there is great uniformity in their interpretations of the general character of the processes involved. Our own work on *Paracopidosomopsis* indicates that the spermatogenesis of this species is very similar, not only as regards the general character of the process, but also as regards many details, to the maturations of the European hornet, *Vespa crabro*, as shown by the work of Meves and Duesberg.

Austin, Texas,
February 6, 1917.

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DESCRIPTION OF FIGURES.

All of the figures are camera drawings made at table level, with the Zeiss 2 mm. lens and No. 12 compensating ocular. The figures thus outlined were enlarged three times by the aid of an enlarging camera, and then reproduced with one third off. They are therefore magnified about 3857.

Plate I.

Fig. 1. Metaphase plate of last spermatogonial division.

Fig. 2. Stage at the beginning of growth period.

Fig. 3. Stage at the mid-growth period, showing the conspicuous cytoplasmic inclusions.

Fig. 4. Spermatocyte with delicate cytoplasmic process.

Fig. 5. Elongation of the cell in preparation for the first spermatocyte division.

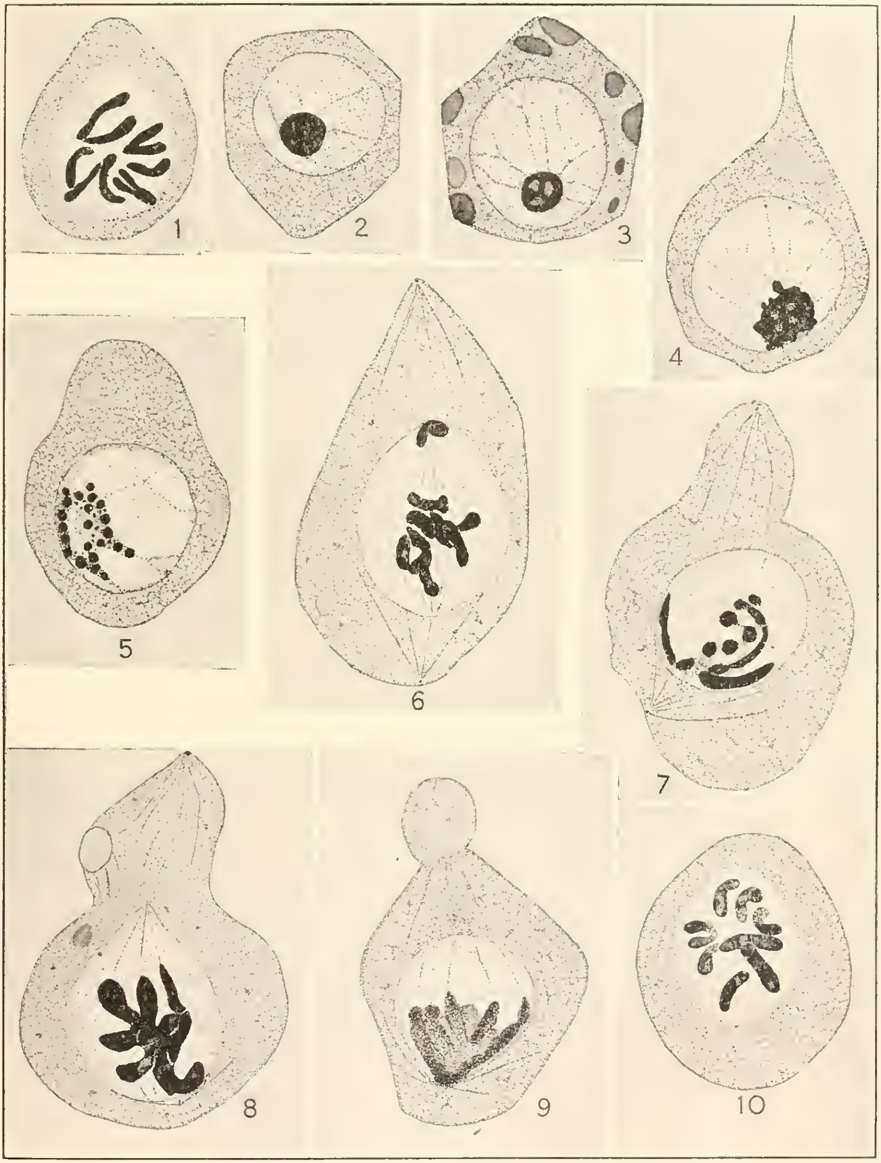
Fig. 6. Stage showing the imperfect intranuclear spindle.

Fig. 7. Stage showing the beginning of the constriction which results in cutting off the cytoplasmic bud.

Fig. 8. A later stage showing the same process.

Fig. 9. Stage showing the end of the first maturation division. The cytoplasmic bud has just been cut off.

Fig. 10. Metaphase plate of the second spermatocyte.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J. T. PATTERSON AND LELIA T. PORTER.

PLATE II.

FIG. 11. Metaphase plate of the second spermatocyte.

FIG. 12. Side view of the second maturation spindle, showing four twisted chromosomes.

FIG. 13. A section through one side of the spindle, showing two chromosomes that have just divided. Parts of two other chromosomes are also present.

FIG. 14. A similar section, showing the complete division of four chromosomes.

FIG. 15. Anaphase stage, showing eight daughter chromosomes passing to each pole.

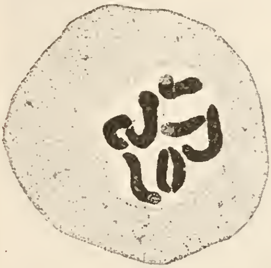
FIG. 16. Late anaphase stage.

FIG. 17. Telophase stage.

FIG. 18. The two spermatids, still connected by means of the interzonal fibres.

FIG. 19. A completely separated spermatid.

FIG. 20. A later stage of the spermatid.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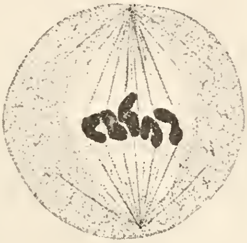
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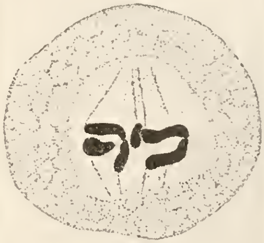
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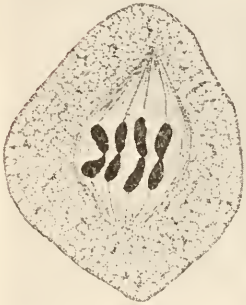
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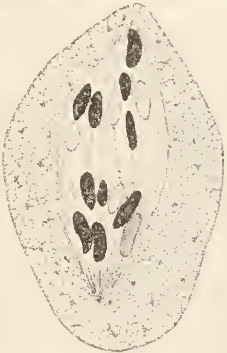
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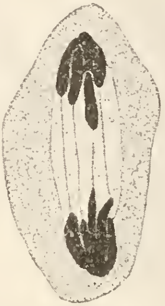
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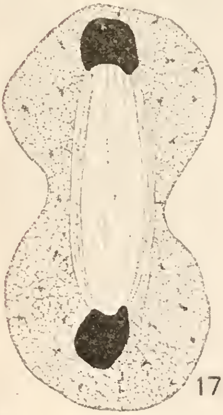
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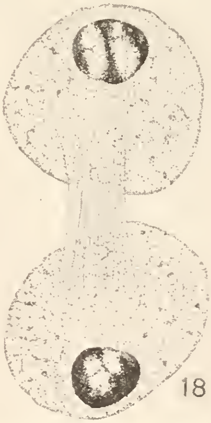
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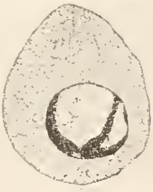
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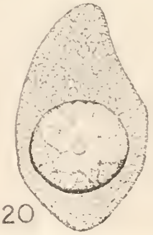
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J. T. PATTERSON AND LELIA T. PORTER.

RHYTHMS AND ENDOMIXIS IN VARIOUS RACES OF PARAMÆCIUM AURELIA.

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In view of the fact that the study of rhythms and endomixis in *Paramæcium* was made chiefly on my long pedigreed race of *Paramæcium aurelia*, it has been suggested by a couple of authors that the reorganization process may be something peculiar to this race, perhaps resulting from long subjection to conditions which preclude the possibility of conjugation. This assumption, however, was rendered exceedingly improbable from the data presented by Woodruff and Erdmann[1] in their original complete paper which showed that "the same reorganization process was resumed in all the lines within a relatively short time after conjugation" was allowed to occur in a subculture of the main race, and also that the phenomenon was observed early in the culture of a race which was secured by Erdmann from Berlin, Germany. Further, Erdmann and Woodruff[2] in a paper on endomixis in *Paramæcium caudatum* stated incidentally that endomixis had been found in two new races of *Paramæcium aurelia* immediately upon their isolation.

The above data more than justify the early conclusion of Woodruff and Erdmann that "this reorganization process is a normal phenomenon and *probably occurs in all races of the species Paramæcium aurelia*," [3] but in connection with other work on *Paramæcium* it has been necessary to employ various races for study and the purpose of the present paper is to record briefly the additional data bearing on the points under discussion. This may be most readily accomplished by considering seratim the graphs of the life histories which show the rhythms and the occurrence of endomixis in the respective cultures. Since the methods of conducting the cultures, plotting the graphs, etc., has been fully described in earlier papers from this laboratory, the reader is referred to them for details of technique. It is only necessary to emphasize here that the occurrence of endomixis has been determined, of course, in each case by the cytological study of animals preserved almost daily from each culture during the periods under discussion.

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[1] *Journal of Experimental Zoology*, Vol. 17, No. 4, 1914.

[2] *Journal of Experimental Zoology*, Vol. 20, No. 2, 1916.

[3] *Ibid.*, 1914, p. 474.

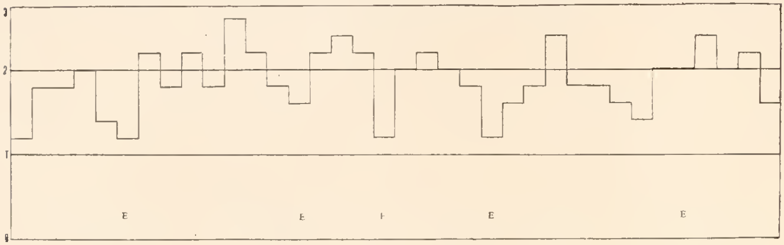

FIG. 1.

Race I. (*Woodruff's Main Culture.*)—Isolated May 1, 1907, at Williamstown, Mass., and carried in pedigreed culture to date (July, 1917). During this period of more than ten years it has attained over 6,000 generations without conjugation. The discovery of endomixis was made on this race and full details

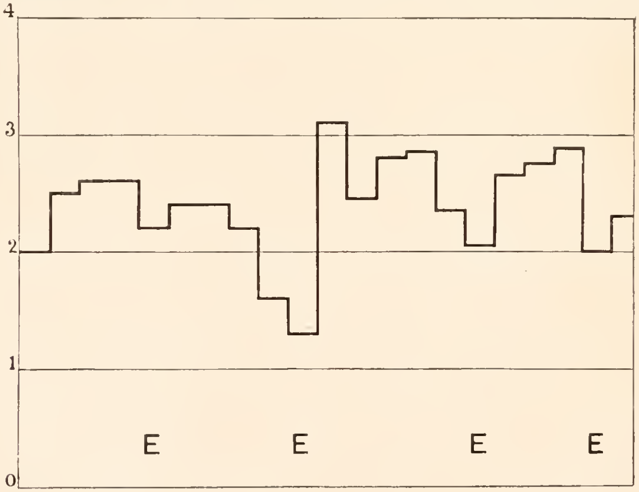

FIG. 2.

have been presented in earlier papers. Fig. 1[1] gives a typical graph of a subculture showing the relation of rhythms and endomixis.

Race II. (*Berlin Race.*)—Isolated by Erdmann in Berlin. For details see Woodruff and Erdmann, 1914.

Race III. (*Oberlin Race.*)—The initial animals of this pedigreed culture was supplied by Professor R. A. Budington, of Oberlin, Ohio. The culture was started October 8, 1914, and was discontinued after nearly two years during which time over 1,000 generations were obtained. Fig. 2 is a graph of the average division rate of the four lines of this culture again averaged for each of the first 21 five-day periods of its life when it was being intensively studied with reference to the occurrence of rhythms and endomixis. The graph shows four rhythms and at the low point between each endomixis occurred. It is to be noted that the reorganization process appeared during the fifth five-day period after the isolation of the culture, thus showing once more that this phenomenon is not dependent upon long subjection to culture conditions for its genesis. The length of the rhythmic periods for this race is also essentially the same as in Race I (cf. Fig. 1).

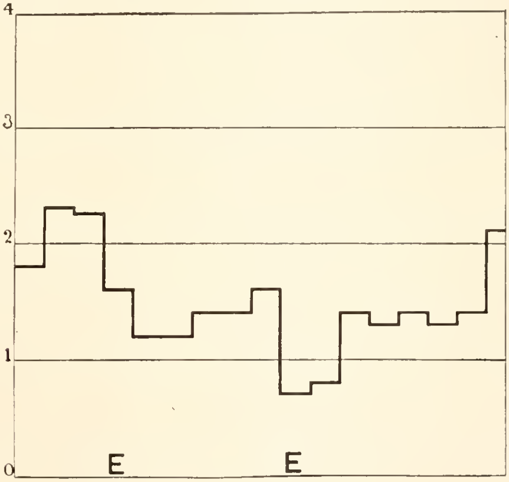

FIG. 3.

Race IV. (*Bryn Mawr Race.*)—The animal which started

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[1] *Ibid.*, 1914, Fig. 17.

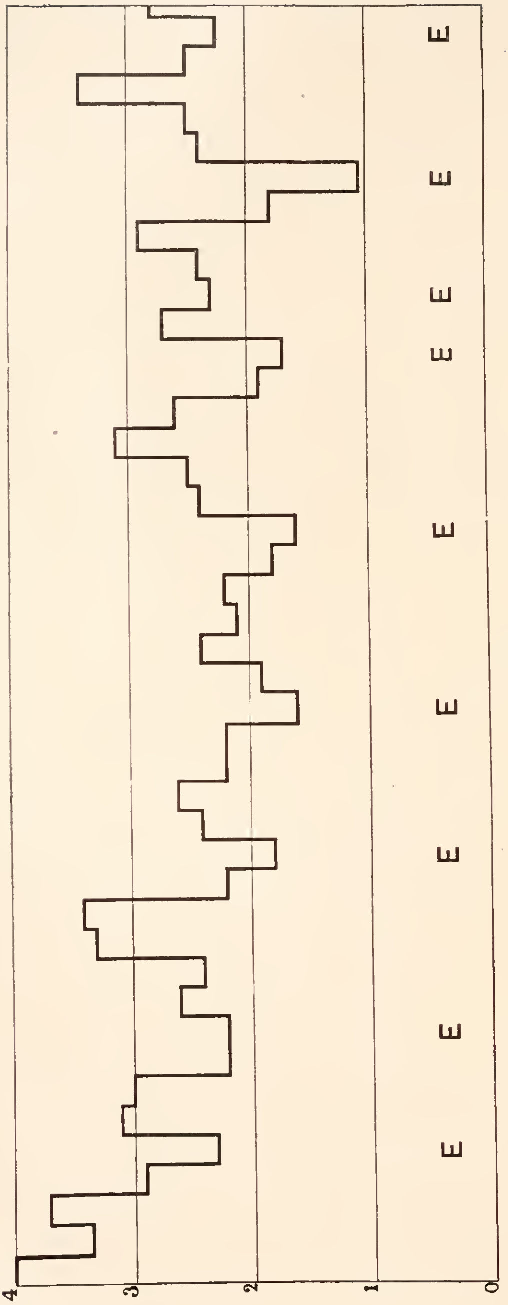

Fig. 4.

this pedigreed culture was isolated on January 7, 1915, from material supplied by Dr. Florence Peebles from Bryn Mawr, Pa. The culture was discontinued at the 650th generation on February 8, 1916. Fig. 3 shows the relationship of rhythms and endomixis during the first eighty-five days of the culture when the animals were being examined for the reorganization process. The results fully substantiate those obtained from previous races. The last inter-rhythm period shown in the diagram is apparently longer than usual—though it is possible that endomixis was overlooked about the 15th five-day period.

Race V. (*Oxford Race.*)—Culture started, from material secured at Oxford, Ohio, on July 16, 1915, and discontinued on August 7, 1916, at the 1,000th generation. The division rate of the culture is shown in Fig. 4 for the first 44 five-day periods of its life. During this time nine rhythms occurred at each of which endomixis was observed. It is hoped that readers who may possess doubts concerning rhythms and endomixis will carefully consider this graph.

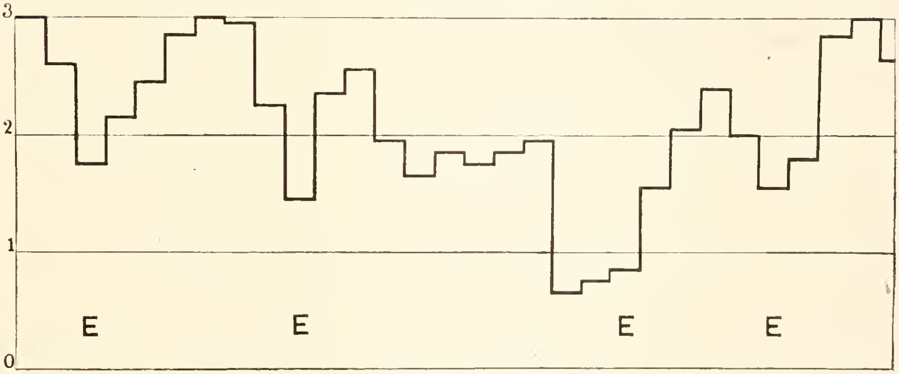

FIG. 5.

Race VI. (*Woods Hole Race.*)—This race was obtained by Dr. G. A. Baitsell at Woods Hole, Mass., and its pedigreed culture was begun on August 11, 1915, and discontinued on January 14, 1916, at the 305th generation. Fig. 5 gives the graph of the division rate of this culture throughout its existence.[1]

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[1] The division rate is plotted consistently one quarter of a division per day too fast for every five-day period, owing to a draughtsman's error. An *E* should be inserted in the first period.

Endomixis occurred as soon as the culture was started and recurred at the usual intervals. The animal was not studied cytologically during the 14th five-day period so that endomixis is not indicated by an *E* in this period on the chart, but from the character of the curve there can be little doubt that an examination of stained specimens would have revealed it.

In a word, then, the data from every culture of *Paramœcium aurelia* (isolated from as diverse localities as Ohio and Germany) which we have studied prove beyond doubt, I believe, that endomixis is a normal periodic phenomenon which occurs in all races of *Paramœcium aurelia.*

Vol. XXXIII. *August, 1917.* *No. 2*

BIOLOGICAL BULLETIN

STUDIES ON THE BIOLOGY OF PARACOPIDOSOMOPSIS.

III. MATURATION AND FERTILIZATION.[1]

J. T. PATTERSON.

INTRODUCTION.

In the second paper in this series (Patterson and Porter, '17) an account of the spermatogenesis was given. It was shown that males reared from unfertilized eggs carry the haploid number of chromosomes in their germ cells, and that as a consequence the first maturation division is abortive, while the second results in producing two equal spermatids, and hence two similar spermatozoa. The purpose of the present paper is to give an account of the maturation of the egg, and of fertilization.

It was originally planned to report on the maturation process both in fertilized and unfertilized eggs, but my series of the latter has proved to be very incomplete, so that the present account is based entirely on a study of inseminated eggs. However, it is highly probable that maturation is identical in the two types of eggs of *Paracopidosomopsis*, as Silvestri ('06) has found to be the case in *Litomastix*.

It is not a difficult matter to obtain material for the study of maturation. The best way is to place a few non-parasitized moth eggs in a closed vessel containing a large number of female parasites. In the course of an hour each host will have deposited in it several eggs of the parasite—sometimes as many as twenty-five or thirty. The host eggs, thus serving as containers for the parasite eggs, can then be fixed as desired. No difficulty has been experienced in cutting the moth eggs into sections from three to five microns thick. Preparations of the entire egg have also been used in the study of maturation. These were made from smears of the host egg.

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[1] Contribution from the Zoölogical Laboratory of the University of Texas, No. 132.

The Freshly Deposited Egg.

The freshly laid egg of *Paracopidosomopsis* is a pear-shaped cell, very similar in appearance to that of *Litomastix*, as figured and described by Silvestri ('06). Different eggs vary considerably in size, and to some extent also in shape. In Fig. 2 is shown a typical egg some time after it had been deposited. The average egg measures about 115 μ in its long axis and 60 μ at its widest diameter.

The egg may be divided into two parts, the anterior portion or pointed end, and the posterior portion or broad end. At the time of deposition there is no line of demarcation between these two parts, but after the beginning of cleavage they are recognized as the polar region containing the polar nuclei, and the embryonal region consisting of the true embryonic cells.

The egg is surrounded by a thin but tough membrane, probably a true chorion. It is also possible that a very delicate vitelline membrane is present, although this is difficult of demonstration.

The contents of the egg consists of a very finely granular protoplasm in which are found a few scattered yolk or oil spherules (Fig. 8). The fertilized egg contains three distinct bodies, the germinal vesicle, the so-called nucleolus, and the sperm (Fig. 1).

The germinal vesicle is a very conspicuous spherical body, mesuring about 19 μ in diameter. Its chromatin is in the form of small stellate bodies. There is also present a small but distinct nucleolar-like body (Fig. 1, *n*). Although the position of the nucleus, as well as that of the nucleolus and of the sperm, is very variable, yet it usually lies toward the pointed end of the cell.

The so-called nucleolus in the eggs of polyembryonic insects has given rise to a great deal of discussion as to its origin and fate. Silvestri ('06, '08), who first described and named this body, thought that it arose at an early stage of the oöcyte from the germinal vesicle, but Martin ('14) and Hegner ('14, '15) have demonstrated that it arises outside the germinal vesicle, and therefore does not represent a true nucleolus. Silvestri ('14) has later admitted the correctness of their conclusions, and proposed the term "oösoma." As to the fate of this body, Silvestri showed that it enters into certain definite cleavage cells, which he believed give rise to germ cells. Hegner has elaborated this idea, bringing it into harmony with his well-known views on germ-line-determinants.

The sperm is invariably found in the posterior half of the egg (Figs. 1, 2, 8), indicating that insemination must take place at any point on the surface of the broad end of the egg. The entire sperm enters the egg (Fig. 8), and soon thereafter forms a small male pronucleus (Figs. 1, 2, 13). There is no evidence that polyspermy ever occurs in the egg of this species, as no egg has ever been found with more than a single sperm in it.

First Maturation.

As in the eggs of many other Hymenoptera, the maturation divisions of *Paracopidosomopsis* do not result in the formation of distinct polar bodies, but instead only the chromatin undergoes division. Preparation for the first maturation division is seen in the appearance of heterotypic or bivalent chromosomes in the early prophase stage. These are sometimes in the form of V's and crosses, and in the clearest cases eight are present (Fig. 7).

The position of the first maturation spindle is very constant. It lies well toward the anterior end of the cell, with its long axis directed toward the center of the egg (Fig. 8). In the anaphase stage there is present a distinct mid-body at the equator of the interzonal or spindle fibers (Figs. 8, 9). At the close of the division the two groups of chromosomes are not reorganized into nuclei; but lie close together surrounded by clear spaces in the cytoplasm.

In only a few cases have I been able to determine accurately the number of chromosomes that pass to either end of the first maturation spindle. In Fig. 9 the outer, or first polar body group contains eight chromosomes, and at the lower end at least seven can be counted. In Fig. 10 the lower or second oöcyte group has eight clear chromosomes. In the upper or first polar body group eight are also present, but they are somewhat massed together. These observations indicate that the first maturation division results in separating the two components of each bivalent chromosome, so that the first polar body receives eight whole chromosomes, as does likewise the second oöcyte.

Second Maturation.

The second maturation occurs soon after the first is completed. The chromatin does not reorganize a nucleus. The first polar body chromatin likewise divides without forming a nucleus.

The second maturation and the division of the polar body may occur simultaneously (Fig. 12), or the polar body division may either precede (Fig. 11) or follow (Fig. 2) the second maturation division. There is therefore no close correlation between the divisions of these two groups of chromosomes.

In Fig. 11 the late anaphase stage of the first polar body division shows eight chromosomes at each end of the spindle. The group of chromosomes lying just below also has eight. This is the second oöcyte group. In Fig. 12 the two division figures are in the late anaphase stage. At the upper end of the polar body spindle (above) six chromatin masses are present, while at the lower end there are eight. The second oöcyte spindle has seven above and eight below. If these two divisions are equational in character, we should expect to find eight chromosomes in each group. Our failure to do so is probably due to the fact that one or more chromosomes in a group may be hidden by some of the other chromosomes.

Fertilization.

As already stated the egg is inseminated by a single sperm, which lies in the lower half of the newly laid egg. Although the entire sperm enters the egg, only the sperm head becomes transformed into the male pronucleus, the formation of which is completed by the close of maturation.

Of the four groups of chromosomes referred to in connection with Fig. 12, the three lying nearest to the surface of the egg represent the three polar bodies, while the one lying toward the center is the oötid group. The fate of the polar bodies will be considered later. The oötid group soon forms a small, condensed female pronucleus, which approaches the male pronucleus with which it eventually fuses. The pronuclei come together in the lower half of the egg. Apparently for a considerable period they lie close together before the actual fusion occurs. In Fig. 13 the two condensed pronuclei lie a short distance apart, with a small aster and its tiny centrosome lying between them. This case is interesting because it is the only one in which I have observed either a centrosome or aster in the eggs of this species.

The pronuclei remain apart while they undergo expansion, the female nucleus always being somewhat larger than the male nucleus (Fig. 15). Upon coming in contact with each other, the pronuclei fuse (Fig. 14) to form a single large conjugated, or cleavage nucleus (Fig. 16). Many different stages of conjugating nuclei have been observed in the preparations, one slide alone showing more than a dozen cases.

Discussion.

These observations show that in the egg of *Paracopidosomopsis* two typical maturation divisions occur, resulting in reducing the sixteen chromosomes of the primary oöcyte to eight in the oötid. It was shown in the second paper of the series that the sperm also receives eight chromosomes. The fertilized egg should therefore have sixteen chromosomes. A study of cleavage divisions shows that this is the case. Fig. 17 is a polar view of a metaphase plate of the first cleavage spindle. It has sixteen chromosomes. In Fig. 18 a side view of an anaphase stage is shown. This is one of the first four blastomeres undergoing division. There are fifteen chromosomes lying toward each end of the spindle, and the sixteenth, just completing its division, lies at the middle of the spindle, on the right.

A study of somatic cells shows that the diploid number of chromosomes is also present in them. Thus the dividing cells of the central nervous system of female larvæ have sixteen chromosomes (Figs. 3, 4). On the other hand, dividing cells from the central nervous system of male larvæ have but eight chromosomes (Figs.

5, 6). This is what would be expected, if males develop parthenogenetically from eggs that have undergone maturation.

AUSTIN, TEXAS,
March 5, 1917.

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DESCRIPTION OF FIGURES.

PLATE I.

FIG. 1. Entire egg from a smear preparation, made 23 minutes after oviposition. It shows the germinal vesicle (*n*), the sperm (*s*) and the nucleolus (*no*). ×720.

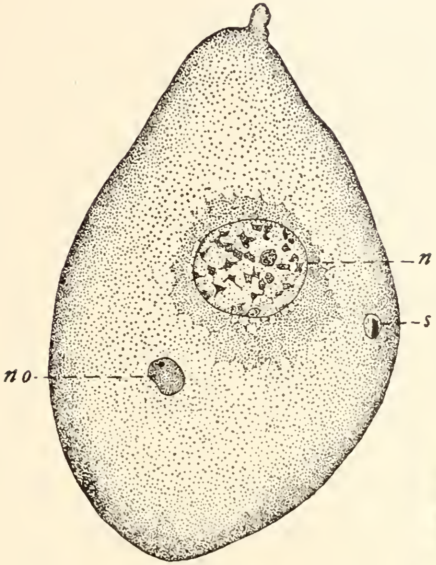
FIG. 2. Entire egg from a smear preparation, showing sperm (*s*), 1st polar body undergoing division (*1 p.b.*), second polar body (*2 p.b.*), egg nucleus (*n*), and nucleolus (*no*). ×737.

FIG. 3. Metaphase plate of dividing cell from central nervous system of female larva. It shows 16 chromosomes. ×3857.

FIG. 4. Same as 3. ×3857.

FIG. 5. Metaphase plate of dividing cell from central nervous system of male larva. It shows 8 chromosomes. ×3857.

FIG. 6. Same as 5. ×3857.



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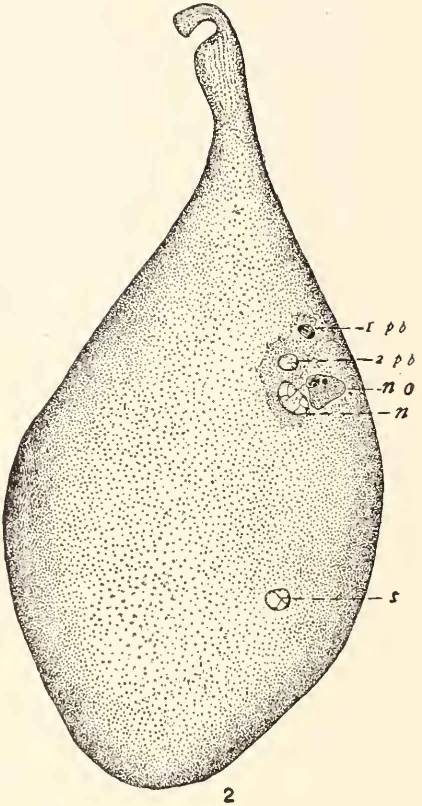
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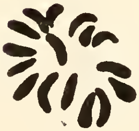

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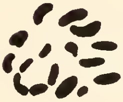

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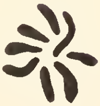

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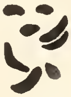

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J. T. PATTERSON.

PLATE II.

FIG. 7. Early prophase of first maturation. The black body close to germinal vesicle is the nucleolus. ×1,240.

FIG. 8. Late anaphase of first maturation. The sperm was drawn in from the next section to the left in the series. ×1,240.

FIG. 9. First maturation spindle. ×1,827.

FIG. 10. First polar body chromosomes above, and chromosomes of the second oöcyte below. ×1,827.

FIG. 11. Anaphase stage of first polar body above, and chromosomes of second oöcyte below. ×1,827.

FIG. 12. Anaphase stages of the first polar body above, and second maturation below. ×1,240.

FIG. 13. Very early stage of the pronuclei, female above, male below. Note tiny aster lying between them. ×1,240.

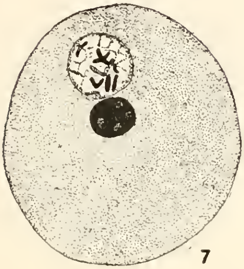
FIG. 14. Fusion of male and female pronuclei. ×1,240.

FIG. 15. Pronuclei, male above, female below. ×1,240.

FIG. 16. Conjugated nucleus. ×1,240.

FIG. 17. Polar view of first cleavage spindle, showing sixteen chromosomes. ×1,827.

FIG. 18. Side view of cleavage spindle of one of the first four cells. There are fifteen chromosomes in each daughter group. The sixteenth chromosome, which has just divided, lies on right side of spindle. ×1,827.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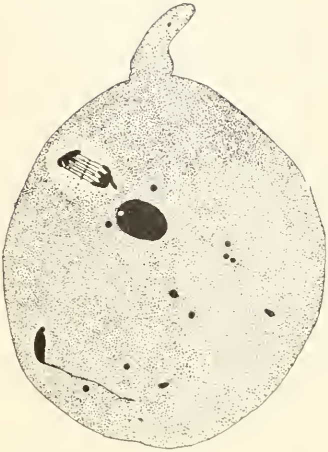
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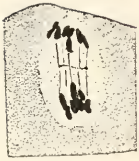
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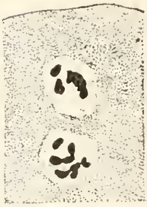
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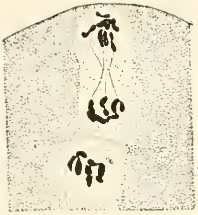
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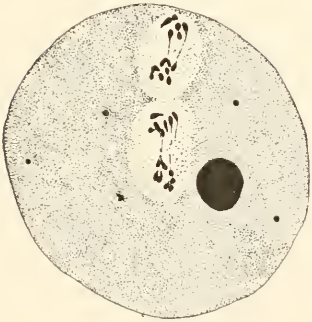
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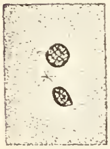
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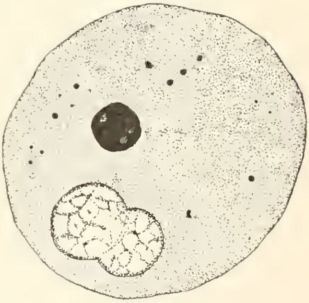
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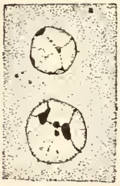
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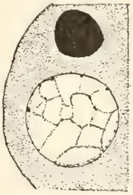
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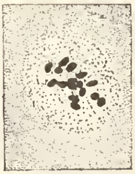
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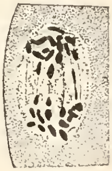
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J. T. PATTERSON.

SOME CHEMOTROPIC AND FEEDING REACTIONS OF PLANARIA MACULATA.

ROSALIND WULZEN.

(From the Rudolph Spreckels Physiological Laboratory of the University of California.)

When a hungry[1] Planarian is placed in a finger-bowl of water a piece of liver has been lying for some minutes, the worm moves about the dish until it enters the diffusing meat-juice. It pauses at once and extrudes the pharynx. This waves about for a few seconds and is withdrawn. Then the worm takes up its course directly toward the meat and, reaching it, begins feeding. The same reaction is seen when the worm is gliding on the surface film and a bit of egg-yolk is floated near it. This preliminary extrusion of the pharynx, although regularly appearing when hungry worms first come into contact with meat-juice, is very transitory. It does not occur again if the worm leaves the juice and reënters it on its way to the meat or if it is placed in an extract of meat and left to wander about there.

If, however, a section is made through the worm behind the cephalic lobes and anterior to the pharynx the result differs. Directly after the cut the worms move about in an active manner though they eventually come to rest. If while still moving they enter a region of diffusing meat-juice they often stop. The pharynx is extended and remains so; feeding begins upon the juice itself and may continue until the body has swollen and become lighter colored as in ordinary feeding. Often the reaction is not so prolonged but it is always more free and persistent than in uncut worms. From this a natural inference would be that such pieces placed directly upon meat would respond with the feeding reaction, and this is true. The posterior portion of any hungry worm will feed when placed upon a fresh piece of liver.

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[1] Worms which had been fasting several weeks were used for these reactions. Well-nourished worms do not furnish good material, as their sensitivity to chemical stimulation is low compared to that of fasting worms.

This result is contrary to that of Pearl,[1] and more extended than that of Bardeen.[2]

A certain degree of independent activity on the part of the pharynx is thus indicated. The same thing could be suspected from the feeding reaction of normal worms. Often a normal worm will come to rest side by side but not in contact with a piece of meat. The pharynx will be extruded and directed toward the meat and will stretch out to reach it to such a degree that the portion of the body to which the pharynx is attached will be pulled out of place, making an indentation on the dorsal surface of the worm. The worm itself will not move at all to help the pharynx, whereas if the pharynx were not extended the chemotropic reaction of the whole worm would carry it directly up to the meat.

There seems to be a twofold chemotropic response, that manifested by the movement of the whole worm toward the meat and that manifested by the movement of the pharynx. Both responses are accurately directed, but when the one is active the other is apparently inhibited. Thus when the stimulation is comparatively weak, the sensitive cells of the skin respond in such a manner that the worm moves directly toward the meat and ordinarily the pharyngeal reflex is entirely in abeyance. When, however, the stimulation is very strong, as upon direct contact with meat or upon the first contact of a very hungry worm with meat-juice, the pharyngeal reflex dominates for a longer or shorter time. When the anterior end of the worm is removed, with it are taken away those portions of the skin which are most sensitive to chemical stimulus. Now a weak stimulus, as that of diffusing juice, calls forth a more pronounced pharyngeal response as though some inhibition were removed.

Some independent chemotropic response seemed so certainly to be located in the pharynx that the question of the activity of the isolated pharynx was raised. If a section is made through the anterior extremity of the pharyngeal sac gentle pressure will force out the pharynx which is now entirely separate from the body. It must be observed that pharynxes so prepared vary as much in sensitivity as do the worms from which they are extracted. But given a sensitive preparation of which any number can be obtained the reaction is as follows: When placed in a dish of water close to a piece of liver, the pharynx elongates actively, its delicate lip is extended into a broad funnel, the walls of which undulate constantly, receiving and being directed by the stimulating substances of the meat-juice. The result is that the funnel is pointed toward the meat, and not only so but the whole pharynx moves forward sometimes several millimeters by a very wormlike series of extensions, contractions and wrigglings until the mouth comes into direct contact with the meat. There is no doubt of the power of the pharynx to orient itself toward the source of diffusing juice and its ability to move toward that source.

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[1] Pearl, R., *Quarterly Journal of Microscopical Science*, 1902–3, Vol. 46, p. 509.

[2] Bardeen, C. R., *American Journal of Physiology* (1901), Vol. 5, p. 1.

The feeding reaction of the pharynx upon reaching the meat is of enough interest to deserve description. Contact of the mouth with the meat causes a wave of peristalsis to pass almost instantly through the pharynx and as a result a mass of food is discharged from the end of the pharynx to which the intestine would normally be attached. Other waves follow in quick succession and the consumed food is discharged at each wave with considerable force. This activity continues for many minutes and results in the accumulation of a surprisingly large heap of food. The shape of the passage through the pharynx is more or less maintained by the masses of food and the little strings of food particles show a tenacity which indicates that the pharynx has added a secretion probably of a mucous nature to the substance passing through it. The experiment can be duplicated with liver extract or yolk of egg dropped carefully on to the bottom of a dish containing water. The pharynx will work its way into the substance and move sometimes a considerable distance in irregular course through it. During the movement it is constantly eating and leaving behind a trail of consumed food. While this reaction is in progress the pharynx bears a remarkable resemblance to a whole organism. The casual observer would immediately call it a living worm. Such entire maintenance of function by an isolated organ is certainly unusual.

THE ACCESSORY CHROMOSOME IN A FROG POSSESSING MARKED HERMAPHRODITIC TENDENCIES.

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Department of Zoölogy, University of Kansas.

Introduction.

In the spring of 1916, the writer, while engaged in determining the effects of starvation upon the development of the germ cells and germ glands of larval frogs, noticed an odd chromosomal body in the germ cells of *Rana pipiens*, which simulated the behavior of an accessory chromosome. Owing to the pressure of other work at that time, the matter was neglected until early in the fall of 1916, when a reëxamination of my material led me to believe that the body I had observed the previous spring was probably an accessory or so-called "sex chromosome."

The present paper is a brief preliminary statement of my observations on the peculiar behavior of this body in the germ cells of *Rana pipiens*.

Literature.

So far as the writer is aware, there is little cytological evidence of the presence of an accessory, or sex determining factor in the germ cells of amphibia. Miss King in 1912 described dimorphism in the spermatozoa of *Necturus maculosus* resulting from unequal chromosomal division. This brief paper seems to exhaust the findings upon this particular subject.

There is a great mass of literature dealing with cytological studies of the germ cells of Urodeles, but with the exception of Miss King's paper, inequalities in the distribution of chromatin have never been reported.

King, '07, failed to find any indications of accessory chromosomes in the germ cells of *Bufo*. So far as I am aware, no one seems to have worked with *Rana pipiens*, though the material is excellent in every respect.

Material and Observation.

In the spring of 1916, April 7, to be exact, the writer collected a large number of *Rana pipiens'* eggs from a string of shallow pools near the University campus. The eggs were allowed to develop in the laboratory. The larvæ resulting were used for various experimental purposes, but many were kept until after metamorphosis. Throughout the spring, numbers of the larvæ were killed at odd times up to the period of metamorphosis, in order to obtain a complete series of stages illustrating the normal course of development of the germ glands. It was in the early maturation stages of the germ cells of the older larvæ, that the body simulating the behavior of an accessory chromosome was first observed.

The germ glands of the larvæ were fixed in Flemming's fluid and also potassium-bichromate-acetic; both fixatives gave excellent results. The sections were cut a thickness of 7.5 μ and stained with iron-alum hæmatoxylin. A counter stain of congo red and orange G was used, but equally good results are obtainable without employing counter stains.

Microscopic examination of the preserved germ glands of the older larvæ revealed the odd fact that the animals were of three kinds: males, females, and larvæ indifferent as regards sex; *i. e.*, hermaphrodites, the germ glands of which contained both male and female cells. Richard Hertwig has fully described sexually indifferent frog larvæ, animals potentially capable of developing into either sex; since his paper first appeared, several other investigators have confirmed Hertwig's findings. In *Rana pipiens* this indifferent condition appears to be fairly common, and is especially marked in some larvæ; the animals retaining their bisexual character, even after metamorphosis, when one or other of the two sexes results.

Further examination of my material showed that in the case of the female larvæ, the maturation processes begin early and usually several weeks before metamorphosis; conversely, in the male, the first maturation changes seldom if ever occur until several weeks after metamorphosis. All stages of the early maturation processes of the germ cells are visible in the glands of female larvæ seven to eight weeks of age.

The oögonial number of chromosomes in the female larvæ appears to be twenty-six. It was possible to make, however, only a few counts, and this number stands, therefore, subject to revision. The male spermatogonial counts will be discussed later. Two oögonial counts were made from a specimen of *Rana catesbiana*, which also gave twenty-six chromosomes as the diploid number. (See Fig. 1.) The size and shape of the chromosomes in the two species of frog appear very similar.

In the post-synizesis stages of the germ cells of the female larvæ of *Rana pipiens*, the chromatin threads which appear from the dense, tangled, heavily staining contraction figure (Figs. 2 and 3), are thick, beaded structures, which have a marked tendency to arrange themselves into the well-known bouquet figures. At this stage all of the chromatin has become arranged in the form of pachytene threads, except a rather large, heavily staining body usually found adhering closely to the nuclear wall (Figs. 4–6). This body is somewhat irregular in shape, though constant in size; the shape usually assumed is that of a triangle, with a minute, thread-like structure at the apex. In the bouquet figures, this chromatin mass orients itself toward one pole of the cell along with the free ends of the pachytene threads (Fig. 6).

Study of a somewhat similar body in the adult male indicates that the body observed in the germ cells of the female larvæ represents probably the accessory chromosomes. At the stage shown in Figs. 4 and 6, the body appears double in most cells. In other cells, for instance Fig. 5, the body is shown as single. Attempts to trace this structure back through the synizesis stage proved futile in my *Rana pipiens* material, owing to certain stains employed by me in connection with a different problem, but in specimens of *Rana catesbiana* larvæ 70 mm. in length, pre-synizesis stages reveal a structure apparently identical with the post-synizesis X-body of *Rana pipiens*.

In female *Rana pipiens*, soon after the synaptic stages of the maturation process occur, the young oöcytes are formed and further study of the *X*-body is impossible in the larval form. Attention was then turned to the germ glands of young male frogs.

Through the kindness of Dr. B. M. Allen, I obtained a number of sections of the testes of young frogs killed several weeks after metamorphosis had occurred. One of these young frogs was a pseudo-hermaphrodite, over a year old. The testes of this animal contained both ripe spermatozoa and large oöcytes. The germ glands, though containing many oöcytes, were true testes; *i. e.*, the animal was a modified male. All stages of the maturation process were visible; in many follicles the oöcytes and ripe spermatozoa occurred side by side.

The sections had been stained with hæm-alum, counter stained with eosin when received by me. This stain serves very well for the maturation divisions, but is not to be recommended for spermatogonial counts, as it renders the outlines of the chromosomes hazy. As a consequence of this, all of my spermatogonial counts were obtained from sections of testes from other animals, stained with iron-alum hæmatoxylin.

Ten or twelve spermatogonial counts gave twenty-five chromosomes as the diploid number. (See Figs. 7–9.) The chromosomes vary greatly in size and shape, from large V-shaped bodies to small straight rods.

In the hermaphrodite specimen a great many prophases of the first spermatocyte division were found. The chromosomes at this stage were undergoing reduction in number, and appeared as thirteen ring and dumb-bell-shaped bodies of varying size. (See Figs. 10–12.) At slightly later stages, the rings assume the dumb-bell shape also, and are connected end to end by fine linin threads. Still later stages show the linin connectives breaking and the chromosomes becoming scattered through the nucleus. There are thirteen chromosomes visible in the cells. Near the end of the early prophase period, twelve of the typical dumb-bell-shaped prophase chromosomes round off somewhat, becoming oval-shaped; one of the dumb-bell bodies, however, retains its shape; this body, because of its peculiar behavior, I have termed the *X*-body. It is not difficult to identify at this stage because of its large size and dumb-bell shape (Fig. 13).

During the early metaphase stages of the first spermatocyte division, the prophase chromosomes, now oval-shaped, except the *X*-body, line up in the equatorial region of the cell to form the metaphase plate. The *X* chromosome takes its position on the metaphase plate along with the other chromosomes. The *X*-body is readily detected at this stage by reason of its marked dumb-bell appearance.

Shortly before the chromosomes split, the dumb-bell-shaped *X*-body migrates to one or other pole of the cell, far in advance of the other chromosomes, and comes to lie at the apex of the spindle close to the centrosome (Figs. 14–19). Lateral views of such spindles, with the chromosomes at the metaphase, are difficult to count; the larger chromosomes obscure the smaller in many instances. Usually, however, twelve obviously bivalent chromosomes can be counted at this stage, in the equatorial region of the cell, just previous to splitting of the chromosomes, and one large, dumb-bell-shaped *X*-body, the accessory, at one or the other pole.

The resulting division separates the twelve bivalents into halves, each half migrating toward its respective pole. As a result of this division, an unequal distribution of chromatin to the secondary spermatocytes occurs; one cell receiving twelve chromosomes plus the dumb-bell *X*, the other cell receiving twelve ordinary chromosomes.

A late anaphase of the first spermatocyte division, showing the *X*-body at one pole of the anaphase, the other pole without the accessory, is shown in Fig. 20.

The dumb-bell *X*-body is very conspicuous in many cells in late anaphase stages. It retains the dumb-bell shape, whereas the remainder of the chromosomes are comparatively small, single bodies. In late telophase stages of the first maturation division, all individuality of the chromosomes is lost; the chromosomes clumping together to form a somewhat irregular crescent. In many such stages the accessory chromosome is easily located by the fact that it is so large that half of it projects from the chromatin mass. It may lie horizontal to the long axis of the cell, resting on the other chromosomes, or at times partly imbedded within their mass.

Three first spermatocyte divisions were observed in which the *X*-body appeared as a single and not a dumb-bell-shaped chromosome. Figs. 21–23 show the single nature of the *X* chromosome. In these cells, the *X* is just half the size it appears in other cells.

At the side of the metaphase plate opposite from the *X*-body in one of the three cells, another single body, evidently the product of a precocious division, was observed. This is shown in Fig. 23. In this cell, the dumb-bell accessory seems to have divided, the halves passing to opposite poles. This explanation may also hold for the other two cells in which the *X*-body appears single, though the other half was not observed. There is a possibility that the single appearance of the *X* may be due to end views of the body, though such a possibility seems slight. The very large size of the *X* would, in my opinion, preclude any such possibility.

The secondary spermatocytes, resulting from the division of one of these three cells, the cell in which the *X*-body divided (Fig. 23), would each receive thirteen chromosomes, each cell receiving half of the accessory; whereas, in the case of the remaining two cells, one half the second spermatocytes would receive thirteen chromosomes, the other half, twelve.

Three other cases of dividing first spermatocytes were observed, in which the typical dumb-bell-shaped *X*-body had migrated very early to one pole of the cell, and at the other pole a large, round chromosome appeared.

The two parts of the *X*-body in one cell, were unequal in size, one being considerably smaller than the other. (See Figs. 24–26.) The single, round chromosomes at the opposite pole from the *X*-body in Fig. 24, judging from its size and shape, appears to be the true half of the accessory or *X*.

The behavior of the chromosomes in these three cells is difficult to explain satisfactorily. It is obvious, however, that the secondary spermatocytes resulting from such divisions would receive unequal amounts of chromatin.

Repeated examination of my material convinces me that the normal distribution of chromatin to the secondary spermatocytes, at the first maturation division, is twelve chromosomes, plus the dumb-bell-shaped *X* to one cell, and twelve ordinary chromosomes to the other.

In regard to the case of those cells just described in which the *X*-body appears to have divided precociously, half passing to each pole in advance of the other chromosomes, it is probable that we are dealing with abnormal divisions. The same is true, of course, of those cells in which the accessory either divided precociously, half passing to each pole, but one half becoming linked with a smaller chromosome or perhaps a chromatoid body (see Fig. 24), or else the halves of the X are unequal.

However this may be, it should not be forgotten that the animal in whose germ cells these anomalies of chromosomal distribution occur, is bisexual; *i. e.*, with the germinal products of both sexes in its glands. There may, or may not be, a connection between such abnormalities of chromatin distribution at the maturation divisions, resulting, presumably, in the production of three kinds of spermatozoa, and the fact that in certain strains of this species of frog, males, females and animals possessing marked hermaphroditic tendencies occur.

It is obvious, however, that the presence of oöcytes in my specimen is not due to any inequality in chromatin sharing of the daughter cells at the maturation divisions, because the oöcytes are formed long before the male maturation period occurs. Such oöcytes may be due to unequal chromosomal division at an earlier period; perhaps in the spermatogonia or even primordial germ cells. In this connection it may be mentioned that in those follicles containing oöcytes, and they are far from uncommon in my specimen, spermatids and mature spermatozoa are also usually found. The two kinds of cells may be found side by side. The fact that the germinal products of both sexes are usually found together in the same follicle suggests that perhaps both oöcytes and spermatozoa may be products of the same cell originally. It might not be amiss to point out here that the oöcytes and spermatozoa found in the same follicles of this animal represent advanced stages in the germ cell cycle, not found in the surrounding follicles. The surrounding follicles contain spermatogonia and prophases of first maturation divisions, stages much younger than spermatozoa and egg formation.

The Second Spermatocytes.

The second spermatocytes resulting from the first maturation division divide at once without an observable period of rest. The telophase of the first spermatocyte division leaves the chromatin matter a solid, somewhat irregular crescentic mass. This mass soon rounds off its angularities and arranges itself along the equatorial region of the cell. The outlines of the individual chromosomes are, for the most part, obliterated at this stage. No further reduction of chromosomes occurs at this division. The dumb-bell-shaped X-body is not difficult to identify in the second spermatocyte at the metaphase. Its identification is rendered easy by its large size and peculiar dumb-bell shape. (See Figs. 27–28.) Part of the dumb-bell usually is seen projecting above the metaphase plate. This is well shown in Figs. 27–28.

All attempts to determine the chromosomal number in secondary spermatocytes in my bisexual specimen proved futile, owing to the hæm-alum stain. The dumb-bell X-body divides along with the rest of the second spermatocyte chromosomes. No attempt was made to trace the further history of the accessory body in the spermatids and spermatozoa. I hope to take up this matter in another paper.

The Chromatoid Body.

A number of investigators of amphibian spermatogenesis have observed and reported the presence of an oval, homogeneous chromatoid body in the cytoplasm of the germ cells.

King, '07, reported it in *Bufo*, and claims it forms the acrosome of the spermatozoon. Herman has found a similar body in the spermatids of *Salamandra*. Recently Bachhuber reported the presence of such a body in the spermatocytes of the rabbit. It has also been reported for other animals.

In *Rana pipiens* the chromatoid body is readily found in the cytoplasm of the secondary spermatogonia. I have never observed it in the primordial germ cells of very young larvæ. This structure is of fairly large size, oval in shape and stains readily. It bears considerable resemblance to an extruded nucleolus, though probably has an extra-nuclear origin. The position of the body in the cytoplasm varies considerably. In some cells it adheres closely to the nuclear wall; in other cells it is found near the periphery of the cell. During spermatogonial division it does not appear to divide, though it occurs among the spindle fibres; actual division of the body was observed but once. (See Fig. 29.) The body is readily distinguished from the chromosomes by its peculiar character, and the position it takes during cell division.

In the primary spermatocytes the chromatoid body is conspicuous in the cytoplasm. Whether it does or does not divide at the first maturation division was not ascertained. In Fig. 24 it is shown passing to one pole, apparently undivided.

I make mention of the behavior of this structure here, in order to disarm, beforehand, the criticism which perchance might arise in connection with this paper, that I may have mistaken the chromatoid body for an accessory chromosome. The origin and fate of this peculiar body in *Rana pipiens* seem worthy of further investigation.

Summary and Conclusion.

1. The oögonial number of chromosomes in *Rana pipiens* and *catesbiana* appears to be twenty-six.

2. The spermatogonial number is twenty-five in *Rana pipiens*.

3. In the synaptene stage of the germ cells of the female *Rana pipiens* larvæ, a chromatin body is found which simulates the behavior of an accessory chromosome.

4. The reduced number of chromosomes in the male of *Rana pipiens* is thirteen.

5. At the first maturation division of the germ cells of a frog possessing marked hermaphroditic tendencies, there is an unequal distribution of chromatin to the daughter cells, the inequality varying in different cells.

6. Perhaps this unequal distribution of chromosomes at the spermatocyte division and the resulting inequality of chromosomal distribution to the spermatozoa may account for the fact that males, females, and animals possessing marked hermaphroditic tendencies are found in this species of frog.

In conclusion, I wish to acknowledge my indebtedness to Dr. B. M. Allen for the loan of several valuable slides and to Dr. W. R. B. Robertson for several excellent suggestions.

April 11, 1917.

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EXPLANATION OF FIGURES ON PLATES I–VI.

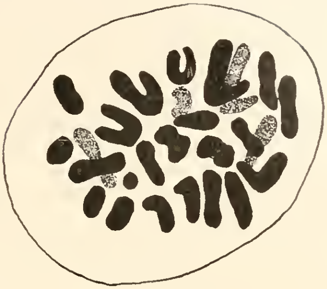
All figures were drawn with the aid of a camera lucida. Spencer 2 mm. apochromat oil immersion objective and oc. 18 used. Drawings are accurate with reference to the nuclear material and chromatoid body. The cytoplasm, however, is represented in the conventional way. *ch*, chromatoid body; *C*, centrosome.

PLATE I.

FIG. 1. Polar view of metaphase plate in dividing oogonia from ovary of *Rana catesbiana*, twenty-six chromosomes are present.

FIGS. 2 and 3. Synizesis stages in young oöcytes of *Rana pipiens* larvæ.

FIGS. 4, 5 and 6. Young oöcytes of *Rana pipiens*, showing the pachytene threads and the X-chromosome adhering to the nuclear wall.



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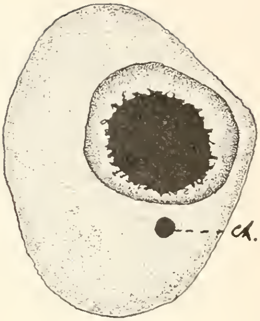

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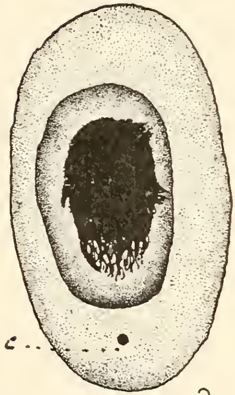

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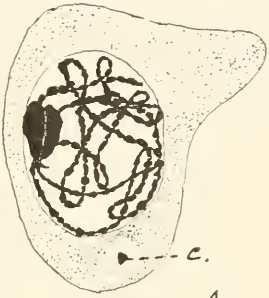

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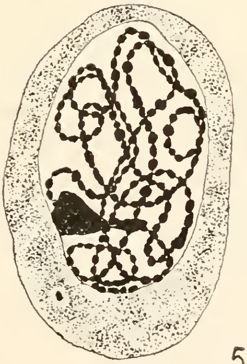

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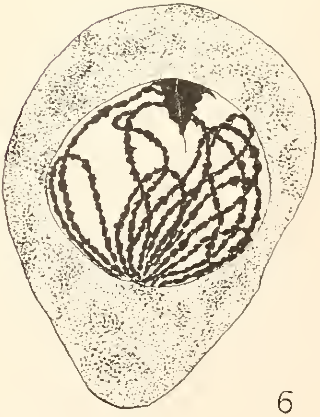

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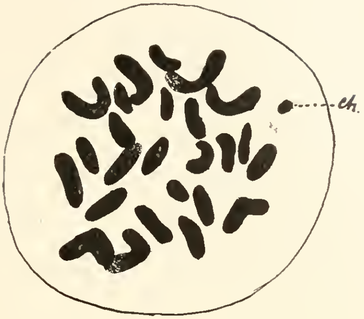
PLATE II.

FIGS. 7, 8 and 9. Spermatogonia of *Rana pipiens*, showing twenty-five chromosomes.

FIGS. 10, 11, 12 and 13. Spermatocytes, showing prophases of the first maturation division.



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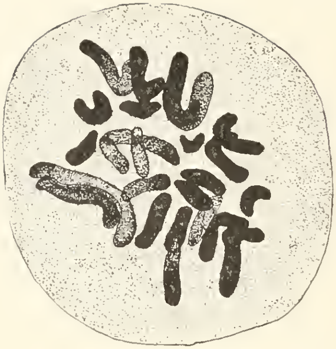

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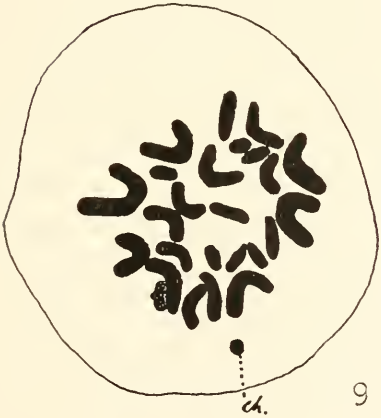

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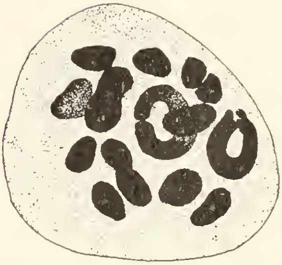

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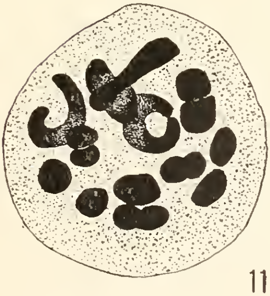

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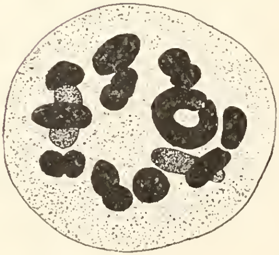

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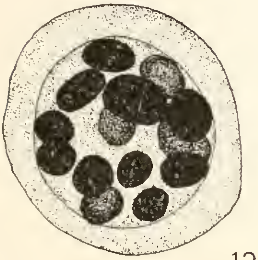
PLATE III.

FIGS. 14, 15, 16, 17, 18 and 19. Spermatocytes, showing first maturation division and the migration of the accessory chromosomes to one pole.



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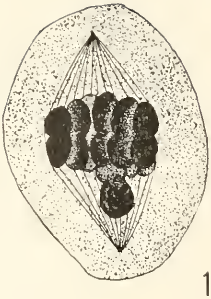

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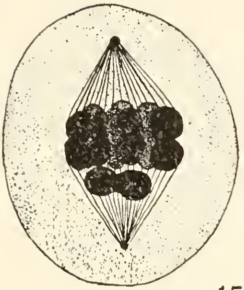

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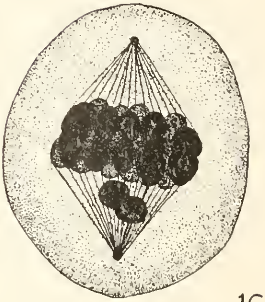

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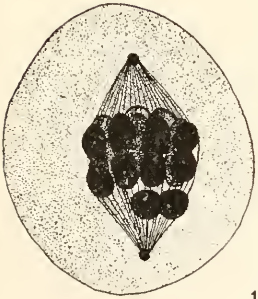

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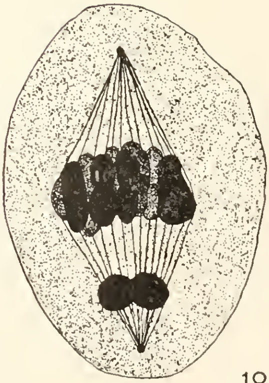

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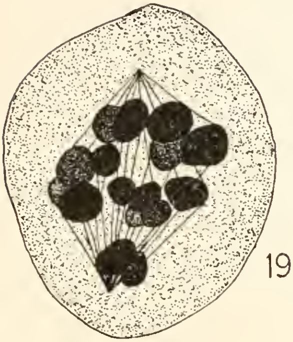
PLATE IV.

FIG. 20. Late anaphase of a dividing first spermatocyte showing the accessory chromosome at one pole.

FIGS. 21, 22 and 23. First maturation divisions showing single nature of accessory chromosome.

FIG. 24. First maturation division showing accessory chromosome at one pole and single round chromosome at opposite pole.



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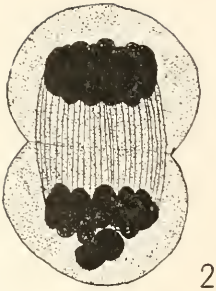

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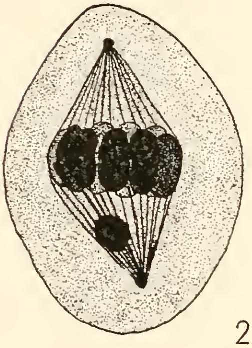

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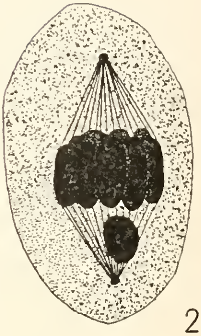

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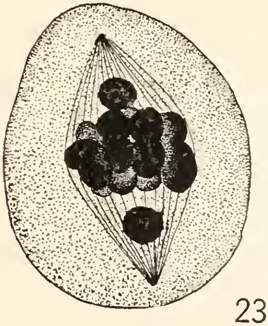

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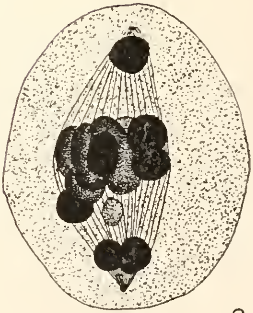

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PLATE V.

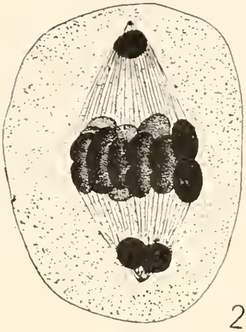
FIGS. 25 and 26. First maturation divisions showing accessory chromosome at one pole of the cell; single chromosome at opposite pole.

FIGS. 27 and 28. Second spermatocytes showing the large accessory chromosome.

FIG. 29. Anaphase of dividing spermatogonium showing division of the chromatoid body.

FIG. 30. Spermatocyte of *Rana catesbiana* with two chromatoid bodies in the cytoplasm.

FIG. 31. Spermatogonium of *Rana pipiens* showing large chromatoid body.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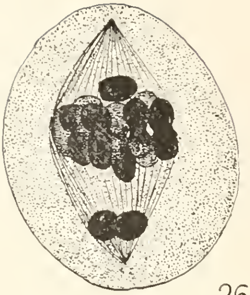
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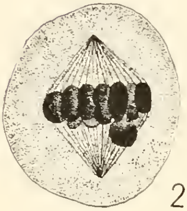
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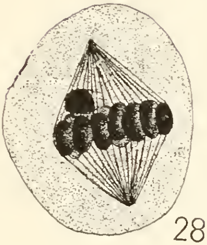
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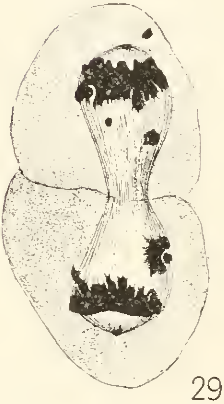
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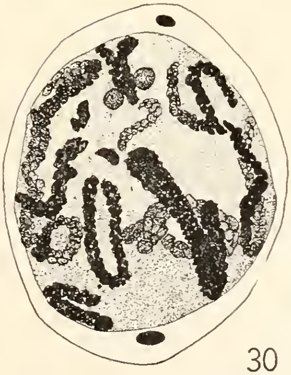
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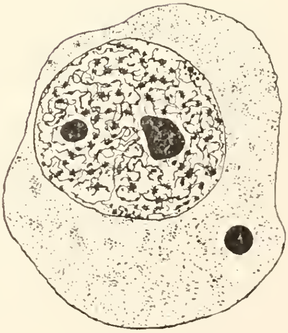
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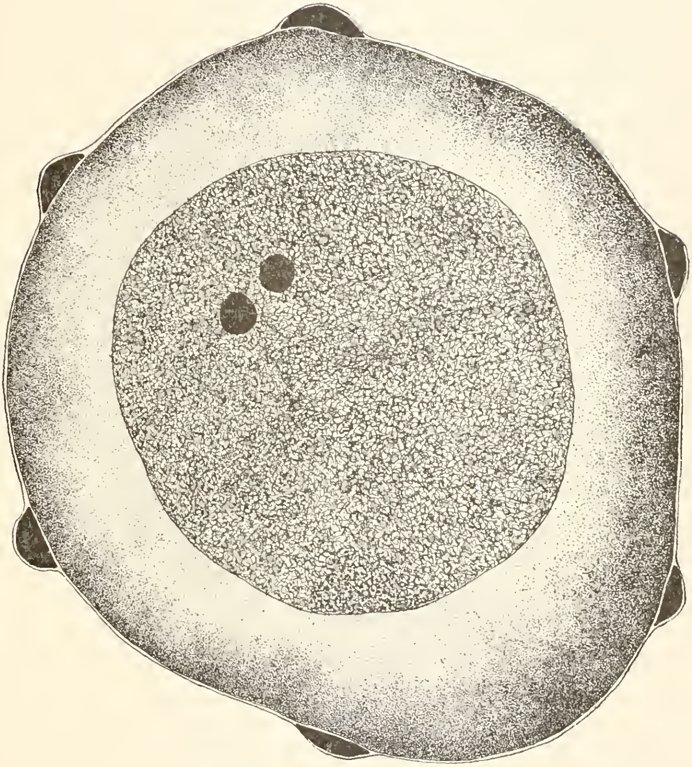
PLATE VI.

FIG. 32. Oöcyte from the testis of young frog.



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THE EXPERIMENTAL PRODUCTION OF HYPOTYPICAL OVARIES THROUGH UNDERFEEDING. A CONTRIBUTION TO THE ANALYSIS OF STERILITY.[1]

LEO LOEB.

In former communications we described a condition of the ovaries in which the follicles become atretic, usually before they have reached medium size. Mature follicles are not produced under these conditions and sterility ensues which persists as long as this condition lasts.[1] Such ovaries we designated as hypotypical. We observed those changes especially in guinea pigs in which the corpora lutea had been burnt at a certain period after ovulation. We must assume that the burning of a part of the ovary caused a "tissue shock" in the remaining part of the ovary which, without killing the follicles, weakened the granulosa cells and thus prevented their further development and caused their early disintegration. In addition we found this change in a number of other animals, some of which had not yet been in heat, and had consistently refused copulation, although their age was such that we might have expected to find them sexually active. In some other cases in which as the microscopic examination showed, ovulation had failed to take place after the conclusion of the last sexual period notwithstanding the degeneration of the corpora lutea, hypotypical ovaries were likewise found. This suggests that this condition may be the cause of long-lasting or perhaps perpetual sterility. This hypotypical condition of the ovaries is of theoretical interest, inasmuch as it represents a state of low developmental energy of an organ that is normally in a condition of constant change. From a practical point of view it is of importance because it can lead to sterility of the gonads. It seemed to us therefore of interest to carry out further experiments in order to throw additional light on the causes of the hypotypical condition of the ovaries. It occurred to us that a common cause might underlie all those cases in which a local injury of the ovaries was not present, and that lack of proper nourishment might be an important factor in this form of temporary sterility. We therefore carried out four series of experiments in which female guinea pigs received the same kind of food as usual, viz., oats and a little grass or green vegetables, but both in much reduced quantity. We shall discuss our experiments in the inverse order in which they were done. In all cases but one both ovaries were cut in serial sections after the completion of the experiment. In one animal only one ovary was cut serially.

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[1] From the Department of Comparative Pathology, Washington University Medical School, St. Louis.

[2] Leo Loeb, *Zentralblatt f. Physiologie*, 1911, XXV., No. 9; *Virchow's Archiv*, 1911, CCVI., 278.

IV. SERIES.

Five guinea-pigs were used in this experiment. (See Table I.)

TABLE I.

IV. SERIES.

Guinea Pig.	Original Weight.	Loss of Weight in Per Cent. of Original Weight.	Period During Which Loss of Weight Occurred.	Result.
A....	294 grams	28%	12 days	Animal weak at end of experiment; about 10–13 days previously ovulation. Hypotypical ovaries of first order.
B.....	309 grams	21% (in 5 days)	6 days	Found dead. Good-sized follicles in granulosa-Degeneration, preparatory to the development of hypotypical ovaries. No ovulation.
*B*1....	288 grams	27%	10 days	Weak, killed. Hypotypical ovaries of second order. Delayed ovulation.
C.....	331 grams	36%	14 days	Weak, killed. Hypotypical ovaries of second order. Delayed ovulation.
D.....	342 grams	34%	14 days	Weak, killed. Necroses in liver. Hypotypical ovaries of first order, delayed ovulation.

Microscopic Examination.

Guinea Pig A.—Had lost 28 per cent. of its weight twelve days after beginning of feeding experiment.

In the ovaries: small and small to medium follicles. In the latter slow disintegration of granulosa cells takes place and connective tissue grows in. Small follicles in various stages of connective tissue atresia; many follicles in the last stage of atresia, with relative prominence of the theca interna. Remnants of old retrogressing corpora lutea: yellowish stained vacuolar cells surrounded by dense hyaline fibrous tissue, traversed by thick-walled blood vessels. In addition we find well-preserved small corpora lutea with capillaries and myxoid connective tissue in center. Mitoses are not present in the corpora lutea. The *uterus* thin, with low cylindrical surface epithelium and small glands. The mucosa is fibrillar. No sign of a cell-layer.

We must assume that ovulation had taken place at least as early as ten days before examination or even somewhat earlier. The structure of the corpus luteum and the absence of all proliferative changes in the uterine mucosa suggest this conclusion. The new corpora lutea remain small as a result of the insufficient food. The follicles develop only to small-medium size and then undergo atresia. Thus hypotypical ovaries are produced. The uterus is thin, atrophic.

Guinea Pig B.—Examined six days after beginning of feeding experiment; had lost approximately 25 per cent. to 27 per cent. of its weight. This animal was found dead; examination several hours after death.

In the ovaries good-sized follicles with granulosa degeneration; good small and small-medium follicles; various stages of connective tissue atresia. Neither a preserved nor a retrogressing corpus luteum present. The *uterus* is thin with the usual low cylindrical epithelium of surface and small glands. Some epithelial cells at top of papillæ show mucoid transformation; fibrillar mucosa. In this case we have to deal with ovaries which are not yet hypotypical, but which apparently undergo some changes tending towards a hypotypical condition; the larger follicles degenerate and the smaller follicles would in all probability fail to develop to their full size. In this animal ovulation had failed to take place throughout a considerable period of time.

Guinea Pig B_1.—Examined ten days after beginning of feeding experiment; had lost 27 per cent. of its weight. *Ovaries* show small and small-medium good follicles. In the latter follicles a slow solution of granulosa sets in. We see some follicles of almost medium size without granulosa, but as yet without connective tissue ingrowth. Follicles in various stages of connective tissue atresia, especially numerous follicles in the last stages of connective tissue atresia with relative prominence of the theca interna. A small yellow fibrous body, the remnant of an atretic corpus luteum is present.

Uterus thin with the usual relatively low epithelium and small glands and fibrillar mucosa. In this case after degeneration of the corpus luteum of the former period, a new ovulation failed to take place and the follicles did not grow to normal size. A hypotypical ovary resulted.

Guinea Pig C.—Examined fourteen days after beginning of feeding experiment; had lost 36 per cent. of its weight. *Ovaries* small, living corpora lutea with small center of fibrous tissue. There is perhaps one mitosis in a Lutein cell. In addition there are fibrous, yellow bodies, the remnants of degenerated corpora lutea. Small and small-medium good follicles. Medium follicles show slow granulosa degeneration. The ingrowth of connective tissue into the follicles is probably somewhat delayed; there are a number of follicles with loss of granulosa and as yet without ingrowth of connective tissue. Various stages of connective tissue atresia of follicles are seen. Atretic follicles in the last stage of atresia and with a large mantle of theca interna are frequent. *Uterus* shows the same character as in the other animals; relatively low epithelium, small glands, fibrillar mucosa. Mitoses nowhere visible.

We may assume that in this case the last ovulation took place very soon after the beginning of the feeding experiment. No trace of proliferation was visible in the uterine mucosa. This indicates that at least ten days had elapsed since the last ovulation, a conclusion in accordance with the character of the corpora lutea. The follicles instead of growing to normal size after ovulation, remained small and underwent premature atresia.

Guinea Pig D.—Examined fourteen days after beginning of feeding experiment; had lost 34 per cent. of its weight. *Ovaries* with small and small-medium good follicles. In the latter slow granulosa degeneration sets in, leading to a complete solution of the granulosa cells with subsequent ingrowth of connective tissue resulting in various stages of connective tissue atresia. Follicles in the last stages of connective tissue atresia form a prominent part of the ovaries. Neither a new corpus luteum nor a remnant of a retrogressing corpus luteum visible. The last ovulation had therefore taken place more than four weeks previous to examination. The *uterus* is thin, with relatively low epithelium and small glands. Much secretion is present in surface epithelium and some gland ducts. The mucosa is thin and fibrillar.

In the four guinea pigs in which ten days or more had elapsed since the beginning of the experiment the ovaries had become hypotypical; while in guinea pig B, which was examined six days after the beginning of the experiment, degenerative changes took place in the large follicles, probably preparatory to the ensuing hypotypical changes. In this animal the relative loss in weight had been almost as marked in six days as in the other guinea pigs in a longer period. It seems therefore that a minimum time has to elapse before the hypotypical condition of the ovaries is established. The latter can be obtained in one of the following two ways: First, after the degeneration of all the good-sized follicles following ovulation, the small follicles fail to reach full size. Before they have grown to medium size, the granulosa cells perish and connective tissue grows in. Second, the large follicles degenerate perhaps at a somewhat accelerated rate without an ovulation having preceded this change, and the small follicles fail to reach medium size. The granulosa of the small-medium follicles does not show the karyorrhexis affecting simultaneously a large number of the granulosa cells and characteristic of the beginning atresia of large follicles; isolated granulosa cells may, however, show karyorrhexis.

In all of the animals with exception of guinea pig *D* ovulation had probably taken place within the last month; and in two animals within the last two weeks preceding the time of examination. We are therefore justified in assuming that at the beginning of the experiment the ovaries had been fully developed in these animals in accordance with their initial weight and age. The lack of a sufficient amount of food however prevented apparently the corpora lutea from reaching full size. It is furthermore probable that toward the end of the experiment the ingrowth of connective tissue into the cavity of the atretic follicles was somewhat delayed. Still the difference in the behavior of granulosa cells and connective tissue is striking. While the former becomes dissolved as a result of the underfeeding, the latter is still active and organizes the cavity of the follicle. The uterus in all cases was thin, and its mucosa did not show any sign of growth. The ova represent a further element still active under these conditions. In the small-medium follicles in process of atresia mitotic and amitotic divisions of the nuclei take place, as well as a division of the superficial part of the egg into cell-like fragments in which however usually nuclei are not visible.

III. Series. (See Table II.)

Fifteen guinea pigs were used in this series, five, weighing between 534 and 380 grams at the beginning of the experiment, had been bred in Missouri; ten younger ones, varying in weight between 378 and 259 grams, were obtained from Iowa. Three of these animals were well fed during the period of the experiment and served as controls. In addition two other control guinea pigs were examined at the beginning of the experiment. We shall first describe ovaries and uterus of the control animals.

1. *Control Animals.*

Guinea Pig No. 3.—Weight Oct. 18, 474 grams. Nov. 11, twenty-four days after beginning of experiment, weight 613 grams. The animal was found pregnant. The ovaries were normal; they contained good follicles of all sizes, large follicles in granulosa degeneration, good mature follicle as well as mature follicles with ganulosa degeneration. Follicles in various stages of connective tissue atresia. Good corpora lutea as well as degenerated corpora lutea (yellow-fibrous bodies). The *mammary gland* was typically proliferating.

Guinea Pig No. 9.—Weight Oct. 18, 286 grams; weight Nov. 2, 375 grams; examined Nov. 4. *Ovaries:* Good corpora lutea, the cavity of which was filled with connective tissue, retrogressing corpora lutea; good small and medium follicles, various stages of connective tissue atresia, especially the later stages are well represented. The *uterus* shows a predeciduomatous cell layer with mitoses in the mucosa. The gland fundi with high cylindrical cells. The *mammary gland* consists of acini with relatively high, well-staining epithelium, but without mitoses, and with rather cellular connective tissue between the acini. We have here to deal with normal sexual organs in an animal at a period about five days after ovulation.

Guinea Pig No. 12.—Weight Oct. 18, 263 grams. Weight Nov. 11, 352 grams. In the left horn of the uterus two pregnancies were found. *Ovaries:* Good corpora lutea; their central cavities are filled with connective tissue; good follicles of all sizes. Follicles with granulosa degeneration; good mature follicles; various stages of connective tissue atresia. *Uterus* is that of pregnancy. In addition to these three guinea pigs, two other guinea pigs obtained from Iowa (1*a* and 1*b*) were examined as controls at the beginning of the experiment.

Guinea Pig No. 1a.—Weight Oct. 19, 378 grams. *Ovaries:* Good corpora lutea; the central cavity is almost completely filled by connective tissue, but a small remnant is still left unorganized. Good small and medium and almost large follicles. Some follicles in the last stages of connective tissue atresia. *Uterus* well developed with a predeciduomatous cell layer in mucosa. Fundi of glands with high cylindrical cells. *Mammary gland:* Acini with good cuboidal epithelium and a few mitoses; cellular stroma. We have here to deal with normal sexual organs in an animal about five to six days after ovulation.

Guinea Pig No. 1b.—Weight Oct. 19, 271 grams. *Ovaries:* Good follicles of all sizes, including large and mature follicles. Much hyperemia around the mature follicles, capillaries in theca interna of these follicles much enlarged. Some follicles in various stages of connective tissue atresia, but relatively few follicles in the last stage of connective tissue atresia. No sign of new or degenerating corpora lutea. The *uterus* showed high cylindrical surface epithelium; gland ducts with high epithelium; gland fundi with lower epithelium. The mucosa is hyperemic and succulent and papillomatous. A few mitoses in surface epihelium and at the opening of the glands. *Mammary gland* very small. A few acini with good cuboidal-cylindrical epithelium with a few mitoses; stroma between acini cellular. The animal was apparently for the first time in her life in the period of heat. We see then that all the control guinea pigs at the beginning and at the end of the experiment were perfectly normal; they were sexually mature and underwent the typical sexual cycle.

2. *Undernourished Animals.*

In order to shorten the description of our findings, we shall from now on designate ovaries in which atresia sets in when the follicles are as yet of small or small to medium size as hypotypical follicles of the first order, and ovaries in which atresia sets in at a slightly more advanced stage of the development of the ovaries, in follicles which have reached or almost reached medium size as hypotypical ovaries of the second order. The uterus is in those cases thin and shows the characteristics described in the fourth series. We shall designate such a uterus as hypotypical.

Guinea Pig No. 1.—Still active at time of examination. Abortion had taken place recently. Ovaries showed corpora lutea with marked vacuolization and thick-walled vessels. The vacuolization affects all parts of the corpus luteum, but especially the periphery. These are the corpora lutea of the preceding pregnancy; following abortion they underwent degenerative changes. Atretic yellow bodies (the remnants of degenerated corpora lutea); good small and medium follicles and only an exceptional good large follicle. The large majority of the large follicles showed granulosa degeneration; follicles in various stages of connective tissue atresia. One almost mature follicle. *Uterus:* Cells of surface epithelium high and large with prominent cytoplasm; glands with rather high epithelium, but with less prominent cytoplasm; many mitoses in surface epithelium; fibrillar mucosa. At one place there are signs that regeneration had taken place and that a decidua had been present. *Mammary Gland:* Large. Acini with good cuboidal epithelium and small lumen. Some colloid in lumen. Some large vacuoles in epithelial cells. No mitoses. Cellular stroma. Some mononuclears and polynuclears in stroma. Beginning secretion following abortion. In this case the underfeeding was probably responsible for the abortion. In the controls otherwise held under the same conditions, but given sufficient food, pregnancy proceeded normally. Ovaries, uterus and mammary gland corresponded to the stage of the sexual cycle of this animal.

Guinea Pig No. 2.—Weight Oct. 18, 464 grams; Nov. 18, 302 grams. Following this date it received a full ration of food for a few days and on Nov. 25 it had regained a part of its loss, weighing now 357 grams. Underfeeding was again resumed. On Nov. 30, it weighed 303 grams and it was examined. *Ovaries:* Two young corpora lutea with dilated capillaries. There is in the corpora lutea a central cavity surrounded by a little peripheral connective tissue. Mitoses are found in the capillaries and in the central connective tissue. Atretic yellow bodies with vacuoles and thick vessels are also present. Small and small-medium good follicles with a very thin layer of granulosa which even around the egg is imperfect. Some mitoses in the granulosa cells. In other small-medium follicles granulosa is lost. Follicles in various stages of connective tissue atresia. The *uterus* shows a high cylindrical surface epithelium, glands with cuboidal cells and a very cellular myxoid mucosa. The *mammary gland* consists of good acini without mitoses; it presents an intermediate character. Ovulation had taken place about 2–3½ days previous to the examination. It is probable that following ovulation the ovaries ceased to develop normally and are becoming hypotypical. The solution of granulosa cells in small-medium follicles suggests such an interpretation. We may assume that probably following the transitory gain in weight a few days before examination ovulation occurred. For a considerable period previous to the last ovulation, ovulation had failed to take place, probably in consequence of the underfeeding. We find therefore only remnants of degenerated corpora lutea, representing the corpora lutea of the preceding cycle. The underfeeding did not prevent mitotic division in various structures, nor did it prevent the development of a slightly predeciduomatous condition of the uterine mucosa.

Guinea Pig No. 4.—The animal was in a dying condition, when examined. Only one *ovary* was examined microscopically. Small and small-medium good follicles; some granulosa degeneration in other small-medium follicles. Various stages of connective tissue atresia; especially many follicles in the last stages of connective tissue atresia, much interstitial gland. No corpus luteum present. *Uterus* showed low cuboidal-cylindrical epithelium of surface and glands without mitoses; fibrillar mucosa. The *mammary gland* consisted of dense fibrous tissue with a few ducts and acini; acini with cuboidal epithelium and without colloid in the lumen. At places the stroma was somewhat more cellular. In this case we can be sure that the ovaries were hypotypical, although only one ovary had been examined. The condition of the uterus makes it certain that a recent ovulation had not taken place. The animal has been markedly affected by the underfeeding, it was in a dying condition at the end of the experiment.

Guinea Pig No. 5.—The animal was found dead. Hypotypical ovaries of the second order. Some medium follicles with granulosa degeneration. Two retrogressing small vacuolar corpora lutea with thick vessels. *Uterus* is hypotypical. In this case ovulation had been delayed.

Guinea Pig No. 6.—Examined in a dying condition. Hypotypical ovaries of the second order. Some almost medium follicles with a thin wall of granulosa. In follicles of almost medium size some granulosa degeneration. No corpus luteum or remnant of a corpus luteum visible. *Uterus* hypotypical.

Guinea Pig No. 7.—Found dead. No noticeable postmortem changes. Hypotypical ovaries of the first order. Small degenerating corpora lutea with vacuolar cells and thick vessels. *Uterus* hypotypical. In this case a new ovulation had not yet taken place, although the corpus luteum of the preceding period was degenerating. The loss of weight in this case had been rapid.

Guinea Pig No. 8.—Found dying. Hypotypical ovaries of the second order. There is present a retrogressing small corpus luteum with vacuolar cells. Uterus hypotypical. Mammary gland shows dense fibrous tissue and small gland ducts without mitoses. Ovulation was delayed.

Guinea Pig No. 10.—Found dead, slight postmortem changes.

TABLE II.

III. SERIES.

Guinea Pig.	Original Weight.	Loss of Weight in Per Cent. of Original Weight.	Period During Which Loss of Weight Occured.	Result.
No. 1..	534 grams	28%	14 days 30% in 24 days	Abortion. Typical cycle, delayed ovulation.
No. 2..	464 grams	23%	33 days 35% in 43 days	Period of refeeding preceded examination. Ovulation with growth in uterine mucosa a few days previous to examination. Ovulation had been suspended for a long period of time. Beginning hypotypical ovaries.
No. 4..	415 grams	31%	14 days	Dying. Hypotypical ovaries of first order.
No. 5..	380 grams	28%	12 days.	Found dead. Hypotypical ovaries of second order. Delayed ovulation.
No. 6..	313 grams	27%	14 days	Dying. Hypotypical ovaries of second order.
No. 7..	302 grams	In 5 days about 9% of the weight lost; had lost some weight in the preceding 10 days	7 days	Hypotypical ovaries of first order. Delayed ovulation.
No. 8..	305 grams	27%	7 days	Dying. Hypotypical ovaries of second order. Delayed ovulation.
No. 10.	292 grams	In 5 days lost 12% of its weight. Died 2 days later	7 days	Found dead. Hypotypical ovaries of first order. Delayed ovulation.
No. 11.	283 grams	26%	7 days	Dying. Hypotypical ovaries of second order. Delayed ovulation.
No. 13.	259 grams	Lost in 5 days 13% of its weight	7 days	Hypotypical ovaries of second order. Delayed ovulation.
Controls.				
No. 3..	474 grams	29%	24 days	Well-developed pregnancy. Normal ovaries.
No. 9..	286 grams	31%	15 days	5 days after ovulation. Ovulation evidently occurred at normal intervals.
No. 12.	263 grams	34%	24 days	Pregnancy. Normal ovaries.
Additional Controls; Examined at Beginning of Experiment.				
No. 1a.	370 grams			About 5–6 days after ovulation. Normal ovaries and uterus.
No. 1b.	271 grams			Animal in first heat. Normal sexual organs.

Hypotypical ovaries of the first order. A small vacuolar corpus luteum. Hypotypical uterus. In this case again delayed ovulation.

Guinea Pig No. 11.—Was found dying. Hypotypical ovaries of second order. Small vacuolar retrogressing corpora lutea. Uterus hypotypical. Delayed ovulation.

Guinea Pig No. 13.—Found dead. Some postmortem changes. Hypotypical ovaries of second order. Small vacuolar retrogressing corpora lutea. Hypotypical uterus.

In this series all the guinea pigs show hypotypical ovaries after a loss of weight of approximately 26–31 per cent. in the course of one to two weeks. In Guinea Pig No. 13 we find in a period of five days a loss of 13 per cent., but in the last two days before death a further considerable loss of weight had in all probability occurred. In 2 cases (Guinea Pigs Nos. 1 and 2) we do not yet find hypotypical ovaries. In the first animal, however, abortion took place toward the end of the experiment. We may attribute the abortion to the lack of a sufficient quantity of food. Abortion is not usual among guinea pigs without a preceding experimental interference. Following the abortion a new ovulation had not yet taken place at the time of examination. In the second animal (Guinea Pig No. 2) ovulation had taken place shortly before the conclusion of the experiment, but there were indications of a beginning hypotypical condition of the ovaries following the ovulation. In these two cases we have in the first place to deal with somewhat heavier animals, and secondly, a slightly smaller relative loss of weight was distributed over a longer period of time. It is probable that the greater initial weight of these guinea pigs caused a greater resistance to the underfeeding; their general condition was less affected by the underfeeding and consequently we find as yet no marked changes in ovaries and uterus. In the five control animals of this series the sexual cycle took its normal course; pregnancy as well as ovulation proceeded normally.

II. Series. (See Table III.)

Five guinea pigs were used in this experiment. Guinea Pig No. 1 served as control, the other four were undernourished and received only water and a few leaves of grass each day. The experiment began June 30, 1916. For briefness sake, only abstracts of the descriptions can be given.

TABLE III.

II. SERIES.

Guinea Pig.	Original Weight.	Gain or Loss in Weight in Per Cent. of Original Weight.	Duration of Experiment.	Result.
No. I, control.	438 grams	A loss of 15 grams	9 days	At end of pregnancy. Normal ovaries and uterus.
No. II.	462 grams	−29%	9 days	Perhaps first change in direction toward development of hypotypical ovaries. Probably abortion a short time previously. Delayed ovulation.
No. III.	454 grams	−38%	9 days	Not hypotypical ovaries, but beginning change tending in that direction. Delayed ovulation.
No. IV.	428 grams	−35% (in 9 days)	10 days	Not yet hypotypical follicles but probably some retardation in the development of follicles. Delayed ovulation. Animal very weak at end of experiment.
No. V..	439 grams	−34% (in 9 days). Then a gain of 24% of the original weight and of 31% of the lowest weight reached July 9	21 days. (9 days deficient and 11 days full feeding)	No mature follicles and delayed ovulation; ovaries otherwise normal.

Guinea Pig No. 1.—Control. In one horn of uterus one embryo: near end of pregnancy. In this case the condition of *ovaries*, *uterus* and *mammary gland* corresponded to the last stage of pregnancy. In the ovaries large and mature follicles were present. The mammary gland was proliferating.

Guinea Pig No. II.—*Ovaries:* Good small and medium follicles, some larger follicles in granulosa degeneration and perhaps one well preserved large follicle. Most follicles seem to degenerate, when in medium size. Various stages of connective tissue atresia. Large vacuolar corpora lutea with thick vessels. Atretic yellow body (remnant of corpus luteum). *Uterus:* Relatively low cuboidal-cylindrical epithelium of surface and glands. Some mitoses in surface epithelium, nowhere else; fibrillar mucosa. *Mammary gland:* Large gland, with acini of various sizes, often filled with colloid material. Fibrous or fibrillar stroma, with small nuclei. Some epithelial cells with vacuoles, indicating slight secretory activity. In this case there is perhaps present a beginning of a change in the direction toward the development of hypotypical ovaries; but as yet the ovaries have not reached this condition. The state of the mammary gland and possibly of the ovaries suggests that an abortion has taken place a relatively short time previous to the examination. The large vacuolar corpora lutea with thick vessels, as well as the condition of the mammary gland, which shows signs of secretion, suggest such a conclusion.

Guinea Pig No. III.—Ovaries: Young corpora lutea. Loose central connective tissue fills the cavity. Atretic yellow vacuolar body with thick vessels (remnant of corpus luteum). Good small, medium and almost large follicles. No large follicles, but a few almost large follicles with granulosa degeneration. A number of small-medium or medium follicles lost their granulosa through a gradual process of solution and form cysts in which the egg is lying free. In other degenerating follicles of this kind connective tissue grows into the cavity. Many follicles in last stage of connective tissue atresia. *Mammary gland:* Good sized, acini with cuboidal epithelium, fibrous stroma. No mitoses. The ovaries are not yet hypotypical, but the condition of the smaller follicles indicates a beginning change in this direction.

Guinea Pig No. IV.—Nine days after the beginning of the experiment the animal was offered a larger quantity of food, but refused to eat it. One day later it was found in a very weak condition and it was examined. *Ovaries:* Corpora lutea vacuolar, with thick vessels; they show beginning degeneration, atretic yellow bodies (the remnants of degenerated corpora lutea). Good small and small-medium follicles. Large follicles show granulosa degeneration. Well preserved large follicles are not present. Follicles in various stages of connective tissue atresia. *Uterus:* Relatively low surface epithelium. Similar epithelium in glands. Fibrillar mucosa without mitoses. *Mammary gland:* small gland in a resting condition with marked development of fibrous tissue. In this case the ovaries are not yet hypotypical, but the lack of good medium and large follicles indicates that some inhibition in the growth of follicles occurred, while the large follicles that had developed previously failed to mature and degenerated.

Guinea Pig No. V.—Weight June 30, 439 grams; July 9, 289 grams. From July 10 on a full ratio of food was given daily and the animal gained in weight. On July 21 it weighed 396 grams; it had not yet regained its original weight. On that date it was examined. *Ovaries:* Contained an atretic yellow body (remnant of a corpus luteum). Good follicles of all sizes, including good large follicles. Large follicles in granulosa degeneration and in various stages of connective tissue atresia. *Mammary gland:* Small intermediate, no mitoses. In this case the ovaries are on the whole normal; but apparently mature follicles did not develop and an ovulation had not taken place for a long period of time previous to the examination, despite the fact that the animal had regained part of its weight.

We find then in this series that hypotypical ovaries did not develop as the result of underfeeding; but in Guinea Pig Nos. II., III., and IV. we find some changes which indicate a tendency toward the development of hypotypical ovaries; either a premature atresia sets in in follicles which are only of medium size, or even smaller, or at least a retardation in the development of large follicles apparently occurred toward the end of the experiment. In all cases mature follicles were absent and ovulation was delayed. In the one animal which received a full ratio of food after a preceding period of underfeeding the follicles likewise failed to mature and ovulation failed to take place during the period in which the animal gained in weight. It is, however, possible that the delay in ovulation was not exclusively due to the undernourishment. In one case abortion had in all probability taken place previous to the examination, probably as the result of the underfeeding. In all these cases the relative and especially the absolute loss of weight had been as great as in the guinea pigs of the III. and IV. series, or it was even greater, and yet hypotypical ovaries had not developed. This result may be due to the greater weight of the animals at the beginning of the experiment. All the guinea pigs weighed between 400 and 500 grams. These animals were therefore less affected in their general health by the lack of sufficient food than the animals in the other series. Only guinea pig No. IV. showed marked weakness. The duration of the experiment was on the average somewhat shorter than in series III. and IV., and it is possible that this factor also has something to do with the result. But it is probable that the greater weight of the animals in the beginning of the experiment is mainly responsible for the lack of the development of hypotypical ovaries.

First Series of Experiments (See Table IV.).—In this experiment the quantity of food given and changes in weight of the animals had not been determined with the same accuracy as in the later series. While we must consider the possibility of errors in the figures for the weight of the animals it is probable that the error on the whole is not considerable. In most cases the amount of food was increased for several days during the experiment and there was therefore a transitory gain in weight during a certain period. Animals *a*, *b*, *c* and *d* had been thyroidectomized some time previous to the beginning of the experiment; the remaining five animals had been normal.

Guinea Pig No. a.—Ovaries: Atretic yellow bodies (remnants of old corpora lutea). Hypotypical ovaries of second order. Hypotypical uterus. *Mammary gland:* Much gland tissue. Resting, intermediate gland.

Guinea Pig No. b.—March 20, weight 650 grams; April 7, 600 grams; April 30, 280 grams. *Ovaries:* Retrogressing corpora lutea, good follicles of all sizes. Large follicles with granulosa degeneration; follicles in various stages of connective tissue atresia. Not hypotypical ovaries; but no mature follicles developed and ovulation was delayed. *Uterus:* With ordinary surface epithelium and glands. Some vacuoles in surface epithelium. Fibrillar mucosa. Some cystic gland ducts at surface.

Guinea Pig No. c.—March 20, 350 grams; April 7, 275 grams; April 11, 175 grams. Found dead. *Ovaries:* With atretic yellow bodies. Hypotypical ovaries of first order. Hypotypical uterus.

Guinea Pig No. d.—Weight March 20, 500 grams. Receives full ratio of food; but loses some weight. April 7, 475 grams; April 10, 400 grams, found dead. Had probably been sick. *Ovaries:* Vacuolar corpus luteum with thick vessels. Small medium and almost large food follicles; some small to medium follicles apparently begin to show connective tissue atresia. *Uterus:* Quiescent with follicular mucosa. *Mammary gland:* Large, resting-intermediate. In this case the loss in weight in the animal caused a condition in the ovaries which is approaching the hypotypical state; there are, however, as yet present some slmost large follicles.

Table IV.

I. Series.

Guinea Pig.	Original Weight.	Change in Weight in Per Cent. of Original Weight.	Duration of Experiment.	Result.
No. *a*..	700 grams	−46%	24 days	Found dead. Hypotypical ovaries of second order. No maturation of follicles. No ovulation.
No. *b*..	650 grams	−57% (In 23 days −53%)	41 days	Killed. Not hypotypical ovaries. No mature follicles; retrogressing corpora lutea. Delayed ovulation.
No. *c*..	350 grams	−50% (In 4 days 21% loss of weight)	22 days	Found dead. Hypotypical ovaries of first order. No new ovulation.
No. *d*..	500 grams	−20% (In 3 days 15% loss of weight)	16 days	Imperfect control. Found dead. Receives a larger quantity of food; almost large follicles in ovaries but some small-medium follicles undergo connective tissue atresia.
No. *e*..	367 grams	Loses 100 grams (27%) in 1st two weeks, then regains weight	38 days	Control; full ratio of food. About 4½ days after ovulation. Normal cycle took place.
No. *f*..	330 grams	−45%	14 days	Died. Hypotypical ovaries of first order. No new ovulation.
No. *g*..	About 420 grams	−58%	17 days	Died. Hypotypical ovaries of first order. No new ovulation.
No. *h*..	About 420 grams	−58%	21 days	Hypotypical ovaries of second order. No new ovulation.
No. *i*..	About 360 grams	−58%	37 days	Hypotypical ovaries of second order. No new ovulation.

Guinea Pig No. e.—Control. Weight March 24, about 365 grams. April 7, 260 grams; received full ratio. May 1, 359 grams; examined. *Ovaries:* Two young corpora lutea with capillaries, with mitoses in lutein and endothelial cells. Atretic yellow bodies. Good small and small-medium follicles; follicles in medium and late connective tissue atresia. *Uterus:* Epithelium in glands high; frequent mitoses in gland fundi. In mucosa predecidual cell layer with frequent mitoses. *Mammary gland:* Large ducts, acini with rather high cuboidal epithelium, very cellular, not fibrous stroma; probably mitoses in acini. This is an animal at a period about 4½ days after ovulation. Mature follicles developed and ovulation took place.

Guinea Pig No. f.—Hypotypical ovaries of first order with small, vacuolar, retrogressing corpus luteum with thick vessels. Hypotypical uterus with a few mitoses in gland ducts.

Guinea Pig No. g.—Hypotypical ovaries of first order; corpus luteum with vacuolar cells and dense fibrous nucleus; probably 15–20 days old. Atretic yellow bodies. Hypotypical uterus. Ovulation had taken place approximately at the time of the beginning of the experiment. The atretic yellow bodies indicate that previous to the experiment the sexual cycle had taken its normal course.

Guinea Pig No. h.—Weight March 24, about 420 grams. April 7, weight 220 grams. April 13, 175 grams. Killed April 14. *Ovaries:* Atretic yellow bodies (remnants of corpora lutea); small and small-medium good follicles. In the small-medium follicles atresia sets in and connective tissue grows into the cavity. A few follicles reach almost medium size and then disintegration of the granulosa and connective tissue atresia set in. Only exceptionally a good medium sized follicle present. Hypotypical uterus; some mitoses in surface epithelium. *Mammary gland:* large. Small acini with or without colloid, with low or medium-sized epithelium and partly vacuolar cells. Stroma fibrillar or fibrous. There may have been secretion in this gland previous to the beginning of the experiment.

Guinea Pig No. i.—Hypotypical ovaries of second order with atretic yellow bodies. Hypotypical uterus; some mitoses in surface epithelium. Guinea pigs *h* and *i* were evidently not so strongly affected by lack of food. They did not die spontaneously at the end of the experiment.

From the results of this series, we may draw the following conclusions: Of the seven animals which were subjected to the low diet, 3 showed hypotypical ovaries of the first order; 3 showed hypotypical ovaries of the second order. In all of these cases ovulation had been prevented. In one case, the ovaries were not hypotypical, although the loss in weight had been just as considerable as in other cases. In this case, however, maturation of follicles and ovulation failed to take place. If we inquire into the condition responsible for these differences in the behavior of different animals, we may suggest that it depends in all probability on differences in the general effect of underfeeding on the animals. Those animals that were alive at the end of the experiment showed a less far-going hypotypical condition than those that had just died at the time of examination. This series shows furthermore that a hypotypical condition may be produced even in a relatively old guinea pig with a considerable initial weight, provided there is a considerable loss of weight extending over a relatively long period of time and a marked influence in the general condition of the animal. If, on the other hand, an animal with a considerable initial weight is less affected generally and its strength is better preserved, throughout the experiment, merely maturation of the follicles and ovulation are prevented from taking place, but a hypotypical condition is not produced. In all those cases in which the ovaries are hypotypical the uterus is thin and shows the resting condition found also in the other series. In this series the loss of weight of the underfed guinea pigs was more considerable than in the other series and the experiment extended over a longer period of time. In the two control animals a hypotypical condition of the ovaries was not found. In one of these the cycle in ovaries and uterus took its normal course; in the other, a loss of weight had occurred, the cause of which remained obscure. While in this case the ovaries do not show a hypotypical condition, some retardation in the development of the follicles had occurred.

CONCLUSIONS.

We used in our experiments altogether thirty-four guinea pigs, eight of which served as controls, while twenty-six were subjected to insufficient feeding extending over periods varying between 33 and 6 days. In the majority of experiments the loss in weight varied between 25 per cent. and 35 per cent. of the initial weight. In some cases the loss was greater, in others less. In the controls there was usually a gain in weight during the experiment, but in two cases there was some loss and in one the loss was as much as 20 per cent. of the initial weight. This animal cannot be considered as a perfect control. In the controls the sexual cycle took its normal course and ovaries and uterus were normal in seven animals. In three of these pregnancy was present which proceeded normally. In several others ovulation occurred during the time of the experiment and the follicles developed in a healthy manner. In the one case, in which the animal lost 20 per cent. of its weight, the ovaries were not yet hypotypical, but some change was noticeable tending in that direction. Of the animals subjected to lack of a sufficient quantity of food the ovaries were hypotypical in eighteen cases. There were differences in the degree in which this condition had developed; while the ovaries of some showed a hypotypical condition of the first order, in others hypotypical ovaries of the second order were present. In the former the development of the follicles proceeded only to that stage in which the follicles were of small-medium size. At this stage atresia set in, while in the second kind a few follicles developed to medium or almost medium size; while others became already atretic at an earlier stage. In no case were large follicles produced in any of these ovaries. In three other cases changes were noticeable in the ovaries which suggested a beginning in the direction toward the development of hypotypical ovaries, while in five animals hypotypical changes were not yet noticeable, but an inhibition of ovulation had occurred owing to the fact that the large follicles failed to mature. We see then that in all the animals a failure of the follicles to mature occurred and in 69 per cent. of the animals the ovaries were hypotypical. In addition pregnancy was found in none of the animals. In one of them abortion had occurred, with certainty and in a second one with great probability, in both cases probably as a result of the underfeeding.

If we now inquire into the causes of the variability in the effects of the underfeeding on the ovaries, our experiments suggest very strongly that in addition to the relative loss in weight two other factors may be of significance, viz., first the length of time during which the loss of weight occurs. It seems that more than six days must elapse, before hypotypical ovaries are found. And secondly, the weight of the animal at the beginning of the experiment is of importance. The more the animal approaches full size, the greater is the difficulty with which the changes in the ovaries set in. They are more readily produced in animals weighing between 300 and 400 grams than in animals weighing over 400 grams. But they can be produced in the latter. The effects on the ovaries are therefore to some extent parallel to the effects of undernourishment on the general condition of the animals. Heavier guinea pigs who suffer the same percentage in loss of weight as smaller animals are apparently very much less affected by it as far as their general health is concerned. It is probable that the greater the portion of the food intake is which the animals have to set apart for growth, the more the portion to be used for maintenance is diminished and it seems furthermore that the effect on the general health and on the condition of the ovaries depends upon the portion available for maintenance. The state of the ovaries determines the condition of the uterus, which is always in a resting, almost atrophic condition in cases in which the ovaries are hypotypical. In a similar manner the condition of the mammary gland depends upon the state of the ovaries. We see then that as a result of underfeeding there takes place an increased destruction of granulosa cells which affects even follicles of small or small-medium size. This degeneration of the granulosa cells at such an early stage of the development of the follicles does not occur in the form of a massive destruction, but here and there cells in the rows of the granulosa adjoining the cavity of the follicles are dissolved. While we may notice cells in which the nuclei undergo karyorrhexis, it is probable that other cells perish without a distinct karyorrhexis taking place. If, on the other hand, a degeneration sets in in the larger follicles in normal ovaries, a large number of granulosa cells are destroyed simultaneously and the nuclei of all these cells show karyorrhexis.

We must therefore assume that if the amount of available food stuffs is diminished below a certain quantity, those cells perish first which are farthest removed from the source of nourishment which is furnished by the capillaries of the theca interna. But the destruction is not limited to the cells adjoining the cavity, but affects in the end the whole granulosa. We see then that under the condition of lack of proper nourishment, a condition which otherwise would occur much later, is observed at an early period of the development of the follicles, and the process of destruction in this case takes place more slowly than in the case of atresia of the large follicles. It is in the small follicles a more chronic, in the large follicles a sudden, acute process. Those granulosa cells in the small and small-medium follicles which survive for a certain time show approximately the same proliferative energy as the granulosa cells of similar follicles in normal ovaries as the investigations of L. S. N. Walsh have shown.[1] We must therefore assume that the remaining cells are fairly healthy and respond to the stimulus to multiply in a way similar to the granulosa cells in normal follicles, while those cells that are markedly affected by the lack of nourishment become dissolved. We have here to deal with a phenomenon similar to the one which we observed in the case of stationary or retrogressing tumors. In our experimental analysis of tumor growth we noticed that in tumors that had ceased to grow mitoses could still be found rather frequently during a certain period following cessation of growth. This observation led us to the conclusion that the stationary or retrogressing condition was brought about not so much through a complete cessation of growth, as through an increased destruction or solution of cells.[2] We see that in the hypotypical follicles the smallest follicles resist, while in the follicles in which a cavity begins to form processes of degeneration set in. We observe the same phenomenon in the destruction of follicles which takes place at the time of ovulation. Here also the largest follicles perish first and the smallest follicles are most resistant. We must assume either that with the growth of the follicles the granulosa cells undergo changes in their constitution, which makes them more sensitive to injurious influences, or that with the growth of the follicles the nourishment becomes more difficult, and that this unfavorable state of the cells makes them more vulnerable, if injurious conditions arise. In case the second interpretation should be correct we would have to assume that the process of degeneration leads auto-katalytically to further destruction of the follicles of the remaining granulosa cells.

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[1] Walsh, L. S. N., *Journal Exp. Medicine*, 1917.

[2] Loeb, Leo, *Virchow's Archiv*, 1902, CLXVII., p. 175; 1903, CLXXII., 345; also E. P. Carson White and Leo Loeb, *Centralbl. f. Bact.*, 1910, LVI., 488.

These observations lead furthermore to the conclusion that the formative stimuli, which call forth cell proliferation, are not identical with the food stuffs on which ultimately the life of the cells depends and which are therefore necessary for cell multiplication, a conclusion in full accord with other facts which may be discussed in another connection. Our previous observations and especially the additional observations of Walsh enable us to state the character of the formative stimulus which acts on the granulosa cells. As far as we can determine this stimulus emanates from the egg, and affects principally those granulosa cells nearest the egg, but to a less extent also the more distant cells. It is probable that this stimulus increases simultaneously with the growth of the follicles from small to medium size. In the medium-sized follicles it reaches a maximum. From now on certain unfavorable factors begin to make themselves felt with increasing severity and they lead ultimately either to a degeneration of the granulosa or to that increased differentiation which is characteristic of maturation of the follicles. Perhaps it is the difficulty in the nourishment of the granulosa—a difficulty which increases with the further enlargement of the medium follicles—which is the factor which counteracts the cumulative action of the ovum and prevents a steady increase in proliferative power of the granulosa cells, and which ultimately leads to their death. Thus may be explained the typical growth curve consisting of an ascending and a descending branch as has been described by Walsh. As we stated above, the ova in the follicles which in the hypotypical ovary undergo a premature atresia, show progressive changes. Thus the same factor that causes destruction of the granulosa cells acts on the eggs as a stimulus. In a similar manner connective tissue cells show activity, migrate into the follicular cavity and thus contribute to the atresia of follicles under conditions in which the granulosa cells perish in consequence of the lack of proper nourishment. We may then conclude that the connective tissue is more resistant to withdrawal of food than the granulosa cells; and this is a conclusion which is borne out by the behavior of the connective tissue under other conditions. This relatively great resistance to lack of food is one of the most important characteristics of the connective tissue which leads to interesting consequences under normal and pathological conditions. While on the whole the connective tissue cells are more resistant than the granulosa cells yet we noticed that under the conditions of ill nourishment prevailing under our experimental conditions, the ingrowth of connective tissue into the follicular cavities was not rarely somewhat retarded. In such cases we see the follicle forming a cyst lined by theca interna in which the ovum lies free or almost free and in which connective tissue has not yet grown.

The atresia of follicles is comparable to a condition which in other organs is designated as cirrhosis. In the case of the atresia of follicles this cirrhotic process is a substitutive one; the stimulus which induces the connective tissue to become active consists in the loss of the granulosa cells which formerly covered the theca interna. As we pointed out on former occasions, we have reason to assume that there exist in addition stimuli of a different character which call forth cirrhotic processes.[1]

From our investigations it follows that in the guinea pig underfeeding prevents maturation of the follicles and thus causes sterility which lasts as long as the effect of the underfeeding is present in the ovary. This lack of maturation we found in all our cases. In addition we found in the large majority of those animals which had become more markedly affected by the lack of sufficient food, especially in those which had not yet reached their full size, a much farther-going deficiency in the development of the follicles, a condition of the ovaries to which we applied the name "hypotypical." The latter state is altogether incompatible with fertility. The problem that is as yet unsolved concerns the length of time during which this condition is present, and whether the ovaries resume their normal life very soon after the quantity of food has again been increased, or whether the hypotypical condition and lack of maturation persists at least for some time after the resumption of feeding. Further investigations will have to solve this problem. Our results may perhaps also have some bearing on the condition of the ovaries during pregnancy. We have shown that in guinea pigs ovulation does not occur during pregnancy unless the corpora lutea have been extirpated. In other species however, and especially in man it seems that during pregnancy a maturation of the follicles is lacking. Our experiments suggest that this may depend upon a relative insufficiency of food available for the ovaries during pregnancy; thus an approach to a hypotypical condition of the ovaries would be produced.

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[1] Loeb, Leo, *Journ. Amer. Med. Ass'n*, 1915, LXIV., 726.

Summary.

Underfeeding, if very pronounced, prevents maturation of the follicles in the ovaries of the guinea pigs in all cases and in the large majority of cases leads to the production of hypotypical ovaries in which atresia of follicles sets in before the follicles have reached medium size. Underfeeding leads to a premature solution of granulosa cells. Connective tissue is more resistant to lack of food than the granulosa. The *uterus* in cases of underfeeding is in a resting or atrophic condition. Thus underfeeding produces at least temporary sterility. Those cells farthest removed from the blood vessels suffer first as the result of underfeeding, die and become dissolved, while in those granulosa cells which remain alive the growth stimulus which in part at least emanates from the ovum causes for a short time a normal cell proliferation. Just as the underfeeding produces more pronounced general effects in younger animals, the effect on the ovaries is likewise more marked in younger animals. Through underfeeding it is, however, possible to produce a hypotypical condition even in old guinea pigs. In those animals which had been pregnant at the beginning of the experiment underfeeding led to abortion.

There exists a noteworthy analogy in the relation of cell proliferation to cell destruction in the hypotypical ovaries and in stationary or retrogressing tumors.

Our results emphasize the distinction between food stuffs and proliferative cell stimuli and they may in addition throw light on the condition of the ovaries during pregnancy.

EXPERIMENTS WITH FEEDING THYMUS GLANDS TO FROG LARVÆ.

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INTRODUCTION.

In 1912, J. F. Gudernatsch published an account of his investigation concerning the effects of feeding various glands of internal secretion to frog larvæ. Among other interesting results observed, was the significant one, that fresh thymus gland, when fed to tadpoles, stimulates the growth processes of these animals and at the same time inhibits the onset of metamorphosis.

In a later communication, January, 1914, this author repeated his previous experiments with thymus feeding, and confirmed his earlier results. For an understanding of the relation between growth and the secretory products of the thymus gland, the importance of the results obtained by this investigator warranted a repetition of his work, which, so far as the writer is aware, has never been questioned. It was with this end in view that the following experiment was undertaken.

Literature.

The experimental researches upon the thymus gland may roughly be divided into two groups: those dealing with the relation between the thymus and bodily growth, and those concerned with the correlation in function of the thymus and sex glands. The literature dealing with the former problem is more extensive than that concerned with the latter.

Effects of Extirpation upon Growth.

One of the first investigators of this problem was Carbone; this experimenter extirpated the thymus of rabbits, and one dog, and found that the animals did not differ in their development from control animals of the same litter.

Another investigator, Ghika, removed the thymus from eight cats and thirteen rabbits; he found that in the first few days there was emaciation and arrest of development which lasted for weeks, although, in some of the animals, the losses were afterwards made good.

Cozzolino removed the thymus from young rabbits weighing 300–400 gm., and in the first two months after the operation was unable to detect any change in their condition.

In a later communication, however, he described crookedness of the legs, arrest of growth, and progressive cachexia in one animal upon which he had operated three months previously.

S. Vicent extirpated the thymus of guinea pigs and did not observe any changes in their condition.

Paton and Goodall found that extirpation of the thymus produced no effect upon the general growth and development of new born guinea pigs.

R. Fischel extirpated the thymus of goats, rabbits and dogs, and obtained negative results. He concluded from his experiment, that thymectomy has no effect upon the development of the subject.

The work of this author has been severely criticized by Basch, who claims that the experimental conditions were inappropriate.

Basch extirpated the thymus of suckling dogs and obtained, striking changes in the growth of his thymectomized animals. The most important growth changes were those affecting the skeleton.

U. Soli found that thymectomy was followed in young rabbits by changes in the development of the skeleton, these changes affecting the hollow, long bones and ribs. This author failed to confirm the results obtained by Basch, with guinea pigs, in which thymectomy is a comparatively simple operation. This would seem to indicate that the symptom complex, observed by Basch following thymectomy in his animals, was probably due to post-operative changes and not connected with the thymus, because in those animals used by Basch, thymectomy is a very severe operation.

Halnan and Marshall extirpated the thymus of young guinea pigs, and found that removal had no influence upon the growth of these animals.

Pappenheimer extirpated the thymus of young albino rats and found that removal had no effect upon body growth and development.

Adler extirpated the thymus of tadpoles and found that these animals suffered no ill effects from the operation. Growth and development was unaffected.

(The work of Gudernatsch has been noted.)

This rather extensive review of the literature was thought justifiable in view of the discrepancy in the results obtained by the various investigators.

Material and Observations.

The following experiment began May 3, 1915, and was completed July 29, 1916. The work in 1915 dealt with the effect of feeding thymus powder to tadpoles, the work for 1916 was concerned with those experiments in which only the fresh gland was employed as food. I shall discuss the work dealing with the fresh gland first, and devote the latter part of this paper to feeding experiments with thymus powder.

Feeding Fresh Thymus Gland.

This work began April 12, 1916. April 2, the eggs of *Rana pipiens* were gathered from a group of shallow pools near the University campus at Lawrence, Kansas, and allowed to develop in the laboratory. One group of eggs was hastened considerably in development by keeping them in a warm room. When the young larvæ had emerged from their gelatinous egg capsules and developed to the free feeding stage, they were placed in glass bowls ten inches in circumference by four inches deep. The tadpoles were covered with ordinary tap water which was changed daily.

When the larvæ had attained a length of nearly 13 mm., one hundred and fifty were selected, and divided into two groups of seventy-five each; one lot was destined for thymus feeding, the other for control purposes. These two lots of seventy-five animals were then subdivided into lots of ten and placed in glass bowls, one lot to a bowl. Both control and experimental larvæ were reared under identical conditions as regards temperature, light and water supply.

A second series of larvæ, one hundred in number and averaging 10 mm. in length, were selected and treated the same as the series above. Fifty larvæ were for thymus feeding and fifty for control purposes. These larvæ were from a batch of eggs gathered a few days later than the larvæ of the first series. Hence, throughout the following experiment, the series averaging nearly 13 mm. in length when started upon the thymus feeding I shall designate as Thymus No. 1, the series averaging 10 mm., at the beginning of the work, as Thymus No. 2.

TABLE I.

APRIL 12.

Length.			
Thymus No. 1, Mm.	Control of Thymus No. 1, Mm.	Thymus No. 2, Mm.	Control of Thymus No. 2, Mm.
12	11	10.5	10
12.5	13	11	9
11.5	12	9	11
13	11	10	9.5
13	12	10.5	10.5
13.5	13	10	10
12.5	13.5	9	9
11	13	11	9.5
12	12.5	9.5	9
12.5	11.5	10.5	10.5
11	14	10	11
11	13	11	11.5
13	13	10	10.5
13.5	14	10.5	11.5
12.5	13.5	9.5	11
11.5	11.5	9	11.5
11	12.5	11	10
12	13	9.5	10.5
13	13.5	10	9.5
13	13	10.5	10
Av. len. 12.25	12.6+	10.1	10.25

The experimental larvæ of both series were each day fed finely shredded bits of the fresh thymus glands of calves 3–12 months of age. Only the glands of young animals were used. The glands were obtained from a nearby slaughter-house, and extirpated by myself.

The control animals were fed fresh beef liver each day. Care was taken to give as nearly as possible the same amount of protein material to the animals in each container and of each group. Both thymus- and beef-fed larvæ ate greedily the food given them.

At the beginning of the experiment twenty larvæ of both the control and thymus-fed groups of larvæ were measured with a millimeter rule, and the average length computed. This seemed a satisfactory method of procedure, considering that all of the larvæ came from the same batch of eggs, and consequently were of the same age.

A very convenient method of measuring the young larvæ is to lay them upon moistened blotting paper. The moisture in the paper prevents injury to the tail and at the same time the larvæ are unable to lash about.

Table I represents the measurements of twenty larvæ of both control and thymus-fed groups April 12, the day the experiment began.

None of the larvæ measured revealed any indications of limb development when examined under the microscope.

Observations of April 20.

When examined upon this date no observable differences in color or activity of the control and thymus-fed larvæ were noticed. None of the larvæ possessed limb buds. Considerable growth, however, had occurred in the larvæ of both groups, but in regard to length and body size, both control and thymus-fed animals were about equal, as Table II indicates.

Observations of April 29.

A careful examination upon this date, of the larvæ of both thymus-fed and control groups failed to reveal any differences in pigmentation, activity, or mortality among the animals. Upon examination under the microscope, tiny limb buds of the posterior extremities were observed on those larvæ of the control group measuring 25 mm. or over. Limb buds were observed on seven of the thymus-fed larvæ measuring 27 mm. and over, in the thymus No. 1 group.

The limb buds of the larvæ at this early stage appeared as two round, blunt projections somewhat anterior and lateral to the anal opening.

TABLE II.

APRIL 20.

Length.			
Thymus No. 1, Mm.	Control of Thymus No. 1, Mm.	Thymus No. 2, Mm.	Control of Thymus No. 2, Mm.
18	18	14	15
19	18.5	15	15.5
18.5	19	14.5	15.5
18.5	19	14	14.5
18	19.5	15.5	16
20.5	19.5	16	14
19.5	18.5	16	14.5
20	17.5	15	15
18.5	18.5	15.5	14.5
18	17	14.5	16
19	18	16	16.5
19.5	19.5	15	15
18	19.5	15	15.5
18	18.5	14.5	16
17.5	18	14.5	15
18.5	20	14	15.5
17.5	17.5	16	15.5
19	18	15.5	16
19.5	18.5	15	14.5
18	20	15.5	15.5
Av. len.....18.65	18.62+	15.05	15.22+

The length of the larvæ in both control and thymus-fed animals was about the same though several slight discrepancies are revealed in Table III. The thymus No. 1 group shows a slight acceleration in growth (average length) when compared with their liver-fed controls. This acceleration was not shown by the thymus No. 2 group. These small growth differences are shown in Table III.

The slight difference in the length of the thymus-fed and control animals may possibly be due to the fact that thymus tissue is somewhat more compact than liver tissue and probably presented greater difficulty for the tadpole in eating. Both kinds of tissue were, however, greedily devoured by the larvæ.

OBSERVATIONS OF MAY 4.

Very faint differences in pigmentation were noticed between the larvæ of the thymus-fed and control groups. The thymus-fed animals were slightly darker than their controls which had a reddish tinge. As regards activity, body-shape and mortality,

Table III.

April 29.

Length.			
Thymus No. 1, Mm.	Control of Thymus No. 1, Mm.	Thymus No. 2, Mm.	Control of Thymus No. 2, Mm.
26 l.b.	25.5 l.b.	23	22
25 "	26 "	22.5	23.5
24.5	25 "	21.5	23
28 "	25.5 "	22.5	22.5
28 "	25 "	22	22
25	25.5 "	23.5	21.5
29 "	30 "	22	21.5
25	25.5 "	21	22.5
26.5	24.5 "	21.5	22.5
27 "	29 "	23	22
27 "	23.5	23.5	23.5
25	24	22.5	23
25	23	22	23.5
24.5	24.5	22	22.5
24.5	23.5	23.5	22
26 "	25 "	23	23.5
25	23.5	22	22
27	24	23.5	22
26	24	22.5	23.5
25.5	23	22	22.5
Av. len. . . . 25.97	24.97[1]	22.45	22.55

no differences between the animals of the two groups were observed. When examined under the microscope, both the control and thymus-fed larvæ had hind limb buds. The limbs had not as yet differentiated into their two primary divisions, nor had the toe points developed. The limb buds of both groups of animals appeared to be of nearly the same size and in the same stage of development. The thymus No. 2 group of larvæ had by this time outstripped the thymus No. 1 group in growth.

The table below shows that the control animals of the thymus No. 1 group averaged, upon this date, somewhat greater length than any of the thymus-fed larvæ. The animals of the thymus No. 2 group were somewhat larger than their controls.

Observations of May 8.

The thymus-fed larvæ upon this date were distinctly darker in color than their controls; no other differences were observed in the two groups of tadpoles. Both controls and thymus-fed

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[1] l.b. = limb buds.

TABLE IV.

MAY 4.

Length.			
Thymus No. 1, Mm.	Control of Thymus No. 1, Mm.	Thymus No. 2, Mm.	Control of Thymus No. 2, Mm.
28 l.b.	29 l.b.	31 l.b.	26 l.b.
30 "	31 "	33 "	33.5 "
29 "	37.5 "	34.5 "	34 "
28.5 "	34.5 "	32.5 "	25.5 "
27.5 "	33 "	33 "	32.5 "
32 "	30.5 "	27.5 "	32 "
34 "	28 "	30.5 "	30 "
28.5 "	37 "	30 "	26.5 "
34.5 "	30 "	35 "	33 "
32 "	33.5 "	32 "	29 "
28.5 "	30.5 "	33.5 "	25.5 "
29.5 "	37 "	37 "	33 "
34 "	33.5 "	31.5 "	30 "
30 "	34.5 "	30.5 "	29 "
26.5 "	32.5 "	30 "	25.5 "
33 "	34.5 "	31.5 "	29 "
31 "	34 "	32 "	27.5 "
27.5 "	32 "	32.5 "	30 "
30 "	30.5 "	34 "	31 "
34 "	33 "	33 "	32 "
Av. len. . . . 30.42	32.8	32.22	29.72

larvæ possessed hind limb buds; these were plainly visible without the aid of a hand lens. The larvæ of both groups of animals had increased in size considerably but the average length of the larvæ of each group varied but little, and such variations are negligible. This is clearly indicated in Table V.

OBSERVATIONS OF MAY 13.

When examined the larvæ of both control and thymus-fed groups had increased in length considerably. All of the tadpoles of both groups had developed hind limb buds and most of the limb buds of the thymus-fed larvæ had differentiated into their two primary divisions and had developed the toe points. The controls seemed somewhat behind the thymus-fed larvæ in respect to the length of the limbs. The pigmentation of the experimental animals was darker than the liver-fed controls.

In other respects the larvæ of the two groups appeared the same. Table VI indicates that the larvæ of all groups averaged nearly the same length. The differences are negligible.

TABLE V.

MAY 8.

Length.			
Thymus No. 1, Mm.	Control of Thymus No. 1, Mm.	Thymus No. 2, Mm.	Control of Thymus No. 2, Mm.
31.5 l.b.	31.5 l.b.	31.5 l.b.	35 l.b.
30.5 "	37 "	33 "	31.5 "
31 "	34 "	34 "	32 "
30.5 "	37 "	32.5 "	33.5 "
30 "	34 "	34 "	30.5 "
36.5 "	33 "	35 "	30 "
40.5 "	37.5 "	34 "	35.5 "
29 "	38 "	33 "	34 "
30 "	33 "	33.5 "	32.5 "
33.5 "	30.5 "	32 "	30 "
33 "	37 "	33 "	32 "
32 "	33 "	37 "	33 "
30.5 "	34.5 "	34 "	31.5 "
33 "	32 "	33 "	30.5 "
29.5 "	33 "	30.5 "	29.5 "
29 "	31.5 "	35.5 "	31.5 "
30.5 "	33 "	32 "	32 "
33.5 "	34 "	31.5 "	32.5 "
32.5 "	32 "	33 "	33 "
34 "	31.5 "	30.5 "	33 "
Av. len. . . . 32.02	33.85	33.12	32.15

TABLE VI.

Length.			
Thymus No. 1, Mm.	Control of Thymus No. 1, Mm.	Thymus No. 2, Mm.	Control of Thymus No. 2, Mm.
37.5 l.b. p.d.[1]	37 l.b.	40 l.b.	44 l.b. p.d.
44 " p.d.	36.5 "	45 " p.d.	45 " p.d.
37 "	42 " p.d.	37 "	39.5 "
33.5 "	33.5 "	37.5 " p.d.	40 "
36.5 "	40 " p.d.	35 "	33.5 "
33 "	35.5 "	33.5 "	45 " p.d.
35.5 "	35 "	33.5 "	40 " p.d.
32 "	37 "	32 "	35.5 "
35 "	38 " p.d.	35.5 "	43 " p.d.
32.5 "	37 "	40 " p.d.	38 "
35.5 "	40 " p.d.	40 "	35.5 "
42 " p.d.	48 "	41.5 " p.d.	39 "
40 "	35.5 "	37 "	38 "
42 " p.d.	33.5 "	38 "	42 " p.d.
43 " p.d.	35 "	34.5 "	42.5 " p.d.
36.5 "	34 "	37.5 " p.d.	41 " p.d.
37 " p.d.	35.5 "	35 "	35.5 "
35.5 "	37 "	34.5 "	34.5 "
34 "	38 " p.d.	37 " p.d.	39 "
39 " p.d.	36.5 "	35 "	37 "
Av. len. 37.05	37.22+	36.95	39.37+

[1] The letters p.d. indicate that the limb buds have differentiated into their two primary divisions.

Observations of May 20.

Examination of the larvæ at this date revealed no difference between the animals of the control and thymus-fed groups as regards pigmentation, body shape, size or activity. The limb buds of both groups had by this time differentiated into their two primary divisions, and had well developed toe points. The size of the limbs of both groups appeared to be the same. The dark pigmentation of the thymus-fed larvæ had gradually faded out, and was no longer visible on May 20. Table VII shows the average lengths of the animals of the experimental and control groups. The average length is nearly the same in all groups. The greatest difference in length between the thymus-fed and control larvæ was about two millimeters.

Table VII.

May 20.

Length.			
Thymus No. 1, Mm.	Control of Thymus No. 1, Mm.	Thymus No. 2, Mm.	Control of Thymus No. 2, Mm.
46 l.b. p.d.	45 l.b. p.d.	44 l.b. p.d.	41 l.b. p.d.
45 " "	45 " "	47 " "	42.5 " "
38 " "	47 " "	39 "	44 " "
46 " "	45 " "	43 " "	39 "
37.5 " "	35.5 " "	41.5 " "	38.5 "
39 " "	37 " "	40.5 " "	41.5 " "
48.5 " "	34.5 " "	45.5 " "	39 " "
37 " "	44 " "	42 " "	40.5 " "
41 " "	46 " "	43 " "	40.5 " "
39 " "	37.5 " "	47 " "	41 " "
39 "	44 " "	46.5 " "	39 " "
42 "	37.5 " "	44.5 " "	41.5 " "
40 " "	46 " "	38.5 "	43 " "
44 "	35.5 " "	40.5 " "	44 " "
37.5 " "	46 " "	39 "	40.5 " "
39 " "	44 " "	38 "	41 " "
38 " "	43 " "	44 " "	39.5 "
39.5 " "	37.5 " "	42 " "	41.5 " "
40.5 " "	38 " "	41 "	38.5 "
41 " "	39.5 " "	39 "	40 " "
Av. len. 40.8	41.37	42.27	40.8

All larvæ which had developed the two primary divisions of the legs in this series, had well-developed toe points.

The larvæ were not measured again until June 8, eighteen days from the date of previous measurement. The larvæ had grown so large that it was considered best to avoid handling them, as much as possible. During the eighteen-day interval only four tadpoles died. One was a control and the other three were thymus-fed animals. The four larvæ became bubbly, *i. e.*, air vacuoles appeared in the region of the lungs under the skin. The larvæ came to the surface and floated on their backs; death usually resulted within a few days. The following table indicates the measurements of the larvæ.

TABLE VIII.

OBSERVATIONS JUNE 8.

Measurements of the Larvæ.											
Thymus No. 1, Mm.			Control of Thymus No. 1, Mm.			Thymus No. 2, Mm.			Control of Thymus No. 2, Mm.		
50	Legs	3.5	54.5	Legs	6.5	47.5	Legs	3	50	Legs	2.5
47	"	3	56	"	10	57	"	4	50	"	2.5
57	"	8	50.5	"	9	55	"	4	50.5	"	2
49	"	2	60	"	10	48	"	3	55.5	"	3
60	"	8	45.5	"	2	44.5	"	2	55	"	4.5
52	"	3	43.5	"	8.5	60	"	10	54.5	"	6.5
48	"	2.5	58	"	8	55	"	4.5	51.5	"	2
55	"	2	58	"	10.5	42.5	"	2	53	"	3.5
45	"	2.5	57	"	6	50	"	3	47.5	"	2
58	"	2	60	"	7	53	"	3.5	55	"	4
49	"	2.5	56	"	5.5	41	"	2	45.5	"	3
60	"	3	48	"	3	58	"	7.5	53.5	"	5.5
51	"	4	52	"	4	50.5	"	5	53	"	5
53	"	3	54	"	4	58	"	5	58.5	"	9
58	"	6.5	45	"	2.5	50	"	2	56	"	6.5
57	"	9	59	"	3.5	55	"	3.5	52	"	4
55	"	6	48	"	2	55	"	4	45.5	"	2.5
50	"	5	49.5	"	2.5	60	"	8	49	"	2.5
56	"	5.5	51.5	"	3	50	"	2	51.5	"	3
60	"	6	53	"	4	62	"	11	53	"	5.5
Av. len. 53.95		4.35	52.95		5.57+	52.6		4.45	52.		3.95

OBSERVATIONS OF JUNE 8.

The larvæ of both control and thymus-fed cultures had grown considerably in the eighteen-day interval between the observations of May 20 and June 8. All of the tadpoles of both groups possessed well-developed limbs, some measuring 11 mm. in length. The limbs of the thymus-fed animals averaged in length about the same as the controls; this was true also of body length, as a glance at Table VIII. will show.

In regard to pigmentation, activity and mortality among the animals of the two groups, practically no differences were observed. The forelimbs of the larvæ had not, as yet, broken through the skin, but showed as slight bulges or swellings under the skin of the pectoral region. The growth of all the larvæ had been retarded, no doubt, by unseasonably chilly weather.

June 10, all of the larvæ of both thymus and liver-fed cultures, on account of their increased size, were removed from their containers and placed in very much larger basins. The new containers were especially constructed sinks, measuring 30 × 18 × 6 inches. Thirty larvæ were placed in each sink, which were so arranged that fresh, well-oxygenated water flowed through them constantly.

By June 20, two of the control larvæ, and one of the thymus-fed group, were in advanced stages of metamorphosis. The forelimbs appeared and the tail was reabsorbed. June 26, these larvæ had completely metamorphosed. From this date on, metamorphic changes appeared in almost all of the larvæ, both of the control and thymus-fed groups. By July 3, six thymus-fed and eight liver-fed larvæ had metamorphosed, and by July 12, most of the larvæ had already metamorphosed, or else were in advanced stages of the process.

The animals were all killed, and their gonads preserved for microscopic examination. Macroscopic examination of the germ glands of both thymus-fed and control animals revealed no observable differences either as regards the size of the gland or proportion of the sexes.

The gonads were fixed in Flemming's fluid and sectioned at a thickness of 7.5 μ. The sections were stained with iron alum hæmatoxylin, counter stained with congo red. Only four of the gonads were examined microscopically, two from thymus-fed and two from control larvæ.

Microscopic examination of the four gonads revealed nothing of interest. Three of the gonads were from female larvæ, and contained young oöcytes undergoing growth. The single male gland contained only spermatogonia and was of normal size. The thymus feeding appeared to have had no effect upon the growth and development of the gonads and the germ cells.

We may say, then, in the light of this experiment, that feeding fresh thymus gland to the larvæ of *Rana pipiens* has no effect upon the growth and metamorphosis of these animals, and the gonads appear to be unaffected by such feeding.

PART II. EXPERIMENTS WITH FEEDING THYMUS POWDER.

The following experiment was started May 3, 1915: The larvæ of *Rana pipiens* were used for the first half of the work. These larvæ had been reared from the egg in the laboratory and were all from one batch of eggs. The feeding work was begun when the tadpoles averaged 28 mm. in length. Most of the larvæ had tiny limb buds of the posterior extremities, at this time, though, as yet, the buds had not differentiated into their two primary divisions. Two series of animals were used, one for feeding work, the other for controls. Both groups were kept under uniform conditions of temperature, light, and water supply. Finger bowls were used as containers, five larvæ to a bowl. Tap water was used to cover them and was changed daily.

The animals of the control group were, each day, fed fresh water algæ, and dried flour paste. This same food was also given to the thymus-fed larvæ, though in smaller amounts in order to obviate any possibility of inanition.

The thymus used was the desiccated glands of sheep prepared by Armour & Co. This powder was mixed with wheat flour in the proportions of three grams of thymus to five grams of flour, enough water was added to make a thick paste which was allowed to dry at room temperature. Pieces of the dried thymus paste about the size of one's thumb nail were finely crumbled and fed to the larvæ. The mixture seemed palatable to the tadpoles for they ate it greedily. The same amount of paste was given each day.

The control animals grew rapidly, and in a few days the limb buds had differentiated into small legs. The development of the thymus-fed larvæ was somewhat slower and, in regard to length, were two or three millimeters behind their controls. On the whole, however, growth in the two series of larvæ was approximately equal, when the fact is considered that the thymus-fed larvæ received less algæ than the controls.

The limbs of the thymus-fed tadpoles differentiated somewhat more slowly than the limbs of the controls, and appeared to be smaller in size. There were no observable differences in the pigmentation of the two groups.

This series of larvæ was kept until June 3, just one month from the date the thymus feeding began. They were measured on this date, the control larvæ averaging 39.5 mm., the thymus-fed larvæ 37.5. The tadpoles were then killed and preserved for miscroscopic examination. It is evident that the thymus feeding did not accelerate growth in the animals of this group. The two millimeters difference between the average length of the control group and the average length of the thymus-fed group is, in animals so variable in their growth capacities as frog larvæ, negligible.

On May 9, a second series of frog larvæ were started upon the thymus diet, appropriately controlled. The controls and thymus-fed larvæ of this series averaged 35 mm. All of the larvæ had hind legs though they were very small, and in most of them the toe points were just appearing. These animals were fed the same kind, and amount of food, as the first series received.

The thymus feeding continued for eighteen days, during this period both controls and thymus-fed larvæ grew rapidly, but as recorded for the first series, the controls differentiated limbs faster.

The length of the two groups of tadpoles was approximately equal, both groups averaging 41.5 mm. eighteen days after the experiment began.

Two other series of larvæ were started later, but the tadpoles were too far along in metamorphosis. No results worth recording were obtained.

August, 1915, I obtained some larvæ of the common bull-frog, *Rana catesbiana*. When found, these tadpoles averaged 32 mm. in length. All except six were used for another experiment. The six remaining larvæ were divided into lots of three each, one for controls, the other for thymus feeding. The nature of the food, and amount fed was the same as that recorded for earlier experiments.

For twenty-one days, this series of tadpoles was fed thymus, and at the end of that period, there was no appreciable difference between the controls and thymus-fed animals either in regard to body length, shape or stage of development. Before the experiment was discontinued the larvæ of both groups of animals were measured. The controls measured in length 41 mm., the thymus-fed animals 39.5 mm.

In this series of larvæ, as in the two previous series, thymus feeding certainly did not accelerate growth.

On November 1, another set of *Rana catesbiana* larvæ were obtained, and all larvæ of 75 mm. in length were selected for thymus feeding. There were twelve 75 mm. larvæ. They were divided into two groups of six each for controls and thymus feeding purposes. The diet of both groups was similar to that given to the larvæ of the previous series.

November 10 the animals of both control and thymus-fed groups were measured. The controls averaged 83 mm. in length; the thymus-fed larvæ 85 mm. The limb buds of both groups were very small. No other differences in the two groups were noticed.

December 9, the larvæ of both groups were again measured. The controls averaged 89.5 mm. The thymus-fed larvæ averaged 90.5 mm. Pigmentation, body shape, were the same in the animals of both groups.

December 23, the animals of both control and thymus-fed groups were again measured. The controls averaged in length 92.5 mm., the thymus-fed larvæ 93 mm. The limb buds of both groups appeared to be of about the same size and stage of development.

The experiment was discontinued at this time.

It was observed in this series that the thymus-fed larvæ averaged slightly more in length than the controls. The differences, however, are very small.

A last series of feeding experiments with powdered thymus was started November 17, 1915. Eighty small tadpoles (species not determined), averaging 17 mm. in length and without limb buds, were brought to the laboratory on this date. Thirty were selected and treated similarly to those of the earlier experiments. These animals were fed thymus from November 17 until December 7. Upon this date the tadpoles of both control and thymus-fed groups were measured. The controls averaged 29.5 mm., the thymus-fed larvæ 30 mm. The disparity in the average lengths of the two series is negligible. The fact to be noticed is that both experimental and control larvæ grew considerably and that both groups possessed tiny limb buds on December 7, when the experiment was discontinued.

Summary of Thymus Powder Experiment.

To sum up the results obtained in the experiments just recorded it may be said that feeding powdered thymus glands to amphibian larvæ of the species *Rana pipiens*, *Rana catesbiana* and *Rana* ——— (species undetermined) does not accelerate growth beyond the normal rate. Some of the observations recorded do, however, indicate that thymus feeding may have a slight inhibitory effect upon limb development. However, in animals so extremely variable in their growth capacities as frog larvæ, to attribute such a slight retardation in limb development to the effects of thymus feeding would be rather unsafe. Aside from this extremely slight retardation in limb development of the thymus-fed larvæ, no results were obtained which would indicate that the feeding of powdered thymus gland to amphibian larvæ has any effect upon growth.

Summary and Conclusion.

To sum up the results of this experiment, feeding thymus gland, either in the fresh state or in the powdered form, to frog larvæ of the species *Rana pipiens*, *catesbiana*, and one undetermined species, does not accelerate the growth processes, or retard the metamorphosis of these animals. The tadpoles seem to develop normally in every respect. The gonads do not appear to be effected by thymus feeding.

These results are at variance with the findings of Gudernatsch. It may be, however, that the species of frog used by this investigator reacts differently to thymus feeding than does *Rana pipiens* and *catesbiana*, thus accounting for the disparity of the results obtained by this investigator and myself.

One point, however, must not be overlooked in this connection, *i. e.*, that the variability of the growth rate in frog larvæ is very great, even in larvæ reared from the same batch of eggs and under similar conditions. Very slight environmental changes are sufficient to bring about fluctuations of the processes of growth in these animals, and even when the changes are reduced to a minimum individual variations are great. I have seen tadpoles of the same age, from the same batch of eggs, reared in the same bowl, that varied as much as 20 mm. in length in five weeks' time. I quote this for what it may be worth, but it serves to indicate the complexity of the factors governing the growth of amphibian larvæ. I cannot but think that the results obtained by Professor Gudernatsch are attributable, in part at least, to some other factor besides thymus feeding.

In regard to the feeding experiments with thymus gland, either in the fresh state or powdered form, one point should not be overlooked, viz., that the growth-accelerating principles of the gland, if there be such, may undergo disintegration or suffer a change by contact with the digestive enzymes in the alimentary tract. Feeding experiments with thyroid glands show that no such changes occur, but possibly the same is not true of the thymus.

In conclusion, I wish to acknowledge my indebtedness to Dr. B. M. Allen, the University of Kansas, for the time and encouragement he has given me in my work.

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BIOLOGICAL BULLETIN

THE FORMATION OF STRUCTURES RESEMBLING ORGANIC GROWTHS BY MEANS OF ELECTROLYTIC LOCAL ACTION IN METALS, AND THE GENERAL PHYSIOLOGICAL SIGNIFICANCE AND CONTROL OF THIS TYPE OF ACTION.[1]

RALPH S. LILLIE.

I. Introduction.

In a recent paper on the nature of the physico-chemical processes underlying the conduction of stimuli in living cells[2] I have called attention to the existence of various significant parallels between the transmission of the effects of local chemical or other alteration in metals and the transmission of physiological influence in organisms. These parallels suggest that processes of a kind related to electrolysis may be concerned in the physiological type of transmission, since such local action in metals (*e. g.*, rusting in iron) is now recognized as a phenomenon of electrolysis, due to the formation of local electric couples between adjoining regions of the surface which differ in composition or solution-tension. In particular the rapidity with which the chemical and other effects at the immediate site of stimulation are transmitted to remote resting regions, in rapidly conducting tissues like nerve, indicates that any process involving an actual transfer of material from active to inactive regions cannot possibly form the basis of the transmission. Some influence of an essentially different kind is indicated; and the type of phenomenon long known to electrochemists as "chemical action at a distance" [3]—in which a chemical change at one electrode of an electrolytic cell instantly calls forth a corresponding change at the other electrode—seems to furnish the closest physico-chemical analogy. In this chemical "distance-action" the transmission is directly dependent upon electrolysis—*i. e.*, chemical change at the contact of electrode and electrolyte, conditioned by the passing of an electric current between the electrodes. The possibility that a process of essentially this type may occur in living cells is not so remote as might seem at first sight, even though conductors of the first class are not present. In many respects the conditions at the boundary between the living cell and its medium are similar to those at the boundary between a metallic electrode and the adjoining electrolyte. Cell-surfaces are electrically polarized; this polarization is subject to change under a variety of conditions; hence local circuits readily arise in cells and nerve-fibers as a result of stimulation or local alteration or injury. These circuits resemble in various essential respects those arising when dissimilar metals in metallic connection are placed in contact with an electrolyte (the usual battery arrangement); thus (1) the potentials can be summed,[1] (2) the potentials vary with the concentration of the electrolyte in essentially the same manner as in the case of metallic electrodes,[2] and (3) a marked degree of polarizability is shown. The inference that chemical changes of the nature of electrolysis may occur where currents enter and leave the cell-surface would seem to be justified by these resemblances. The existence of such a condition would go far to explain many hitherto obscure types of physiological transmission, besides the conduction of stimuli—for example, the transmission of formative or integrative influence in the phenomena of regeneration, form-regulation, growth and development. The general influence of electric currents upon cellular processes would also appear in a clearer light; it is well known that formative and other metabolic processes, as well as activities like contraction and nerve-conduction, are profoundly influenced by such currents. There are also grounds for believing that in the restoration of the normal resting condition of cells, after any kind of activity involving breakdown of material, a process of chemical and structural reconstitution or reorganization takes place, and that in this process electrical factors play an important part.[1] Finally it should be noted that the physiological effects at anode and cathode are typically contrasted in a manner which inevitably recalls the contrast between the two electrodes in electrolysis; and while this resemblance does not prove that processes of electrolysis enter in producing the physiological effect—since the current may have other kinds of polar action—it undoubtedly favors such an interpretation.

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[1] From the Laboratory of General Physiology, Clark University.

[2] *Amer. Journ. Physiol.*, 1916, Vol. 41, p. 126.

[3] *Cf.* Ostwald's article, "Chemische Fernwirkung," *Zeitschr. physik. Chem.*, 1891, Vol. 9, p. 540.

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[1] *Cf.* Brünings, *Pflüger's Archiv*, 1903, Vol. 98, p. 241.

[2] Macdonald, *Proc. Roy. Soc.*, 1900, Vol. 67, p. 325. *Cf.* also Loeb and Beutner, *Biochem. Zeitschr.*, 1912, Vol. 41, p. 3.

The similarity of certain types of electrolytic deposit to vegetative growths has long attracted the attention of chemists. This is especially the case with the so-called lead-trees, tin-trees, etc., obtained when salts of these metals undergo spontaneous electrolysis in contact with baser metals; thus when a piece of zinc is suspended in a solution of tin chloride or lead acetate the metal of the salt is deposited upon the zinc in a tree-like branching form; this metallic deposit grows outward through the solution because each portion of metal as deposited forms a cathodic surface which itself becomes the site of a chemical change similar to that at the original surface of deposition. An analogy to organic growth is evident here; in both processes certain substances are selectively removed from solution, chemically transformed, and deposited to form a definite type of structure. A further resemblance between such a process of electrolytic accretion and true organic growth is that in electrolysis material may be deposited from solutions of *all* degrees of concentration. In this respect the extension of a metallic cathode in a solution of its salt presents a closer analogy to organic growth than does the increase in size of a crystal in a supersaturated solution, since in organic growth the abstraction of food-substances from solution and their transformation into structural material or living protoplasm may also take place from very dilute solution.

Structures having a remarkably close superficial resemblance to plant growths and other cell-structures are also formed under certain conditions (partly described below) from metals undergoing local chemical or electrolytic change in which insoluble precipitates are formed. The rusting of iron is an example of this general type of phenomenon, although here the deposits of oxide, beyond showing a tendency to spread, *i. e.*, to act as centers for the deposit of new oxide, do not as a rule show structural peculiarities definitely suggesting an organic type of growth. Certain other inorganic precipitates, however, such as the ferricyanides of the heavy metals, especially when formed in the presence of protective colloids like gelatine and egg-albumin, may be deposited in a filamentous or vesicular form and give rise to structures which are strikingly similar, both in morphological constitution and in the conditions of their formation, to definite cellular or other organic growths. In the present paper I propose to describe a number of methods by which these structures may be readily formed; and also certain methods of accelerating or inhibiting their rate of formation, which appear to be closely analogous in their fundamental features to the conditions controlling growth-processes in plants and animals. Since these artificial growth-structures are beyond any question produced by local processes of electrolysis, it seems justifiable to regard the various parallels described below as affording strong support to the above view that processes of a similar nature are intimately concerned in the formation and deposition of structural and other materials in living organisms.

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[1] *Cf.* my recent paper in *Amer. Journ. Physiol.*, 1917, Vol. 43, p. 43; see pp. 56–7.

II. Experimental.

The formation of filamentous growths from an electrolytically formed precipitate may be most readily demonstrated as follows. A small piece of ordinary iron, *e. g.*, a nail or tack or piece of wire, is placed in a 2 per cent. egg-albumin solution (fresh egg-white diluted six times and filtered) containing 2 per cent. potassium ferricyanide (K_3FeCy_6). Within a few seconds the blue precipitate of ferrous ferricyanide is seen to form locally at various spots on the surface of the metal; the precipitate is deposited chiefly in the form of slender filaments which rapidly increase in number and length until within fifteen to thirty minutes the entire surface of the metal is covered with a blue-green filamentous hypha-like growth. After a few hours some of the thicker filaments may be several centimeters long, usually extending in a straight or sinuous course over the surface of the solution. They are hollow, unbranched, of smooth contour, and exhibit considerable coherence and flexibility; these properties, however, as well as the rate of growth, vary with the content of the solution in albumin and ferricyanide. The rate of formation also varies with the character of the metal, being in general slower with pure than impure specimens of iron; it is also greatly influenced by the contact of other metals, as will be described in detail below.

The actual process of formation presents a striking spectacle under low powers of the microscope. Within a few seconds the precipitate appears upon the surface of the metal in scattered regions, at first as minute rounded vesicles each enclosed by a thin precipitation-membrane; slender cilia-like filaments then push out rapidly into the solution; these steadily elongate, many reaching in a few seconds a length of 50 microns or more. In most cases growth ceases abruptly at a length of a small fraction of a millimeter; this is usually the case with the more slender formations; the broader filaments may continue to elongate by terminal growth until they reach in many cases a length of several centimeters. At their earliest appearance the filaments strongly suggest organic growths like cilia, and what is still more remarkable is the fact that they often exhibit trembling or wavy or in some cases quite regularly rhythmical to-and-fro vibratory movements. This phenomenon is also observed with the similar filamentous growths of zinc and copper ferricyanide, especially the latter, and will be considered further below. Since in living cells cilia typically arise from locally modified regions of the surface protoplasm ("basal bodies") which serve as centers of rhythmical activity as well as of growth, this behavior of newly formed precipitation-filaments increases the impression that the conditions of formation of these artificial structures are of the same fundamental nature as those of actual cell-structures.

Other metals which form insoluble ferricyanides may give rise in the above solution to filaments of a similar type, differing characteristically in their structure, appearance, and rate of formation from those of iron ferricyanide. Small pieces of zinc form highly characteristic structures of this kind, especially if in contact with carbon or a nobler metal (Ag, Hg, Pt, Cu, Fe). Pieces of pure zinc, without other metal, form filaments in the above solution much more slowly than iron, presumably because of the more homogeneous structure of zinc and the relative absence of regions differing sufficiently in solution-tension (and hence in potential) to form efficient local couples; but when in contact with a noble metal the rate of formation is rapid. Copper in contact with platinum or carbon also forms filaments in ferricyanide solutions containing sodium chloride in sufficient quantity; for reasons that will appear below, the presence of this or a similar salt is necessary to the process. On the other hand, certain metals which readily form insoluble ferricyanides do not deposit the precipitate in filamentous form; this is true of aluminium, lead, tin, and (in part) manganese. Pieces of aluminium and lead in contact with copper or platinum wire form precipitates rapidly in the above solution; but the deposit merely collects in the form of more or less coherent or vesicular masses at various regions of the surface without forming filaments. Apparently only those metals whose ferricyanides form semi-permeable precipitation-membranes can form filaments. Under the above conditions tin forms a slight and non-coherent precipitate; with manganese some formation of filaments is usually observed, but most of the precipitate is deposited in amorphous form. The following observations have been made chiefly with filaments of iron, zinc, and copper ferricyanide. The more minute structural details of these formations will not be described at length in the present paper,—both in order to save space and also because the experiments themselves are so simple and the materials so accessible that anyone can readily make these observations for himself. The following description will therefore be confined to the more general features of structure and the essential conditions of formation.

Conditions of Formation of Filaments.—In pure solutions of K_3FeCy_6 (2 to 4 per cent. in distilled water) the formation of precipitate is slight and gradual. Pieces of iron or zinc remain for hours in such a solution without perceptible change. The addition of a little soluble alkali salt, however, *e. g.*, NaCl, at once leads to a rapid formation of precipitate. The growth of filaments in gelatine or egg-white solutions containing K_3FeCy_6 is in fact dependent upon the inorganic salts associated with the protein. Apparently salts must be present whose anions form soluble compounds with the filament-forming metal; the reason for this requirement will become clear when the process of filament-formation is described in detail. The following experiment shows that the rate of filament-formation varies directly with the proportion of chloride (or similar salt) present in the solution of ferricyanide. A small iron nail (one centimeter long) was placed in each member of a series of solutions of 4 per cent. K_3FeCy_6 containing progressively decreasing proportions of NaCl, viz., 4, 2, 1 per cent., etc., down to 1/128 per cent. An abundant precipitate of iron ferricyanide was formed within ten minutes or less in all solutions containing 1/16 per cent. NaCl or more, and the more rapidly the higher the proportion of NaCl. With 1/128 per cent. NaCl the precipitate formed gradually; while in the entire absence of NaCl none was perceptible after several hours. Similar experiments with pieces of zinc in contact with copper wire gave the same result.

The presence of egg-albumin or gelatine, *i. e.*, an emulsoid colloid capable of having a "protective" action upon finely divided insoluble material, greatly favors the formation of coherent filaments from iron and zinc; with copper this condition is less important. Thus in a solution of 1 per cent. K_3FeCy_6 in distilled water containing a trace of HCl ($n/200$–$n/400$) an iron nail rapidly forms precipitate, but this is largely separated in the form of a loose non-coherent deposit and only partly as filaments. In a similar solution containing 2 per cent. egg-albumin relatively little amorphous precipitate is formed, and the filaments are more numerous, regular, and coherent. Similar conditions hold to an even greater degree for the formation of zinc filaments; in simple aqueous solution the zinc ferricyanide is deposited chiefly in amorphous form or in the form of coarse, brittle, and irregular tubular structures; while with protein present an abundance of slender hypha-like filaments results. This type of phenomenon is undoubtedly of much biological interest. In the absence of the protective colloid the crystals of precipitate unite as they separate out to form coarser aggregates of relatively slight coherence; while in its presence each particle of precipitate is apparently at once coated with an adsorbed film of modified protein which serves to bind the particles together and favors the formation of delicate and regular structures. In organisms the deposition of inorganic precipitates like calcium carbonate or phosphate in the formation of skeletal structures (bone, shell, etc.) takes place under essentially similar conditions; the particles are thus deposited in finely divided form, and their arrangement to form definite and regular structures is facilitated. This influence of albumin or gelatine upon the character of precipitation-membranes and similar structures has been investigated by Quincke;[1] he also finds that the colloid modifies very essentially the character of the structures formed.

A certain minimal concentration of K_3FeCy_6 is also necessary for the formation of coherent filaments. In lower concentrations the precipitate tends to separate out in non-coherent form, producing irregular or amorphous deposits. Thus in experiments with iron nails placed in a series of solutions containing 2 per cent. egg-albumin and 1 per cent. NaCl, with varying concentrations of K_3FeCy_6, it was found that while from a 0.8 per cent. solution of K_3FeCy_6 the precipitate separated chiefly in filamentous form, from a 0.4 per cent. solution it was deposited only in small part as filaments but chiefly as an amorphous mass; in 0.2 per cent. very few filaments were formed, and none in more dilute solutions. The same general result was found in a second similar series with a lower concentration of NaCl (1/8 per cent.). Similarly while zinc readily forms filaments in an albumin solution containing 0.25 per cent. K_3FeCy_6, in a 0.13 per cent. solution only a few filaments are formed. In order that coherent structures should be formed the concentration of soluble membrane-forming salt must exceed a certain well-defined minimum.

Structure and Manner of Formation of Filaments.—The precise structure of the filaments varies according to the nature of the metal, the presence or absence of protective colloids, and the concentration of the ferricyanide and other salts present. The larger filaments are typically hollow or tubular; it is only the shorter and slenderer structures that appear to be solid, and even here the case is doubtful, since filaments that appear solid on ordinary examination often show a distinct double contour when examined more closely. The probability is that all filaments are hollow, at least when first formed, and that each consists essentially of a solution containing a salt of the metal and enclosed by a semi-permeable precipitation-membrane. The mode of formation about to be described indicates this clearly. The appearance of the mass of filaments covering a piece of metal that has lain for some time in the solution is strikingly vegetative or fungoid in character. The special character or "habit" of the growth varies with the different metals; in the case of iron and copper the filaments are usually more uniform in appearance and have a smoother contour and straighter course than in the case of zinc; with the latter metal the growths are more irregular and varied and have a characteristic granular appearance due to adhering coarser particles of precipitate. The appearance of such growths is often remarkably hypha-like in character; thus a thin strip of zinc about two centimeters long with a copper wire bound about one end, placed in a watch-glass containing a 2 per cent. solution of K_3FeCy_6 in dilute egg-white, developed in the course of eighteen hours a most complicated feltwork of winding and interlacing hypha-like filaments, covering the whole strip from end to end. Numerous tubular filaments extended to the surface of the solution and along the surface, in some cases for three or four centimeters; many such surface-filaments had undergone curious modifications suggesting efflorescences or spore-capsules, *e. g.*, becoming enlarged at their terminations to form stalked vesicular or bladder-like formations with thin walls. Such a mass of modified filaments irresistibly suggests a vegetative growth.

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[1] Quincke: *Annalen d. Physik*, 1902, 4te F., Vol. 7, p. 646.

Study of the mode of formation of these tubular filaments under the microscope reveals a somewhat unexpected complexity of conditions. Elongation is not by basal growth, *i. e.*, by deposition of precipitate at the surface of the metal where the metallic ions enter solution, but is always *terminal*, the tube advancing through the solution by the deposition of precipitate at its extremity; this process is accompanied by a flow of solution outward through the tube. Filaments of iron ferricyanide show most clearly the nature of the whole process. In a 2 per cent. solution of K_3FeCy_6 in dilute egg-white containing a little NaCl (0.25 per cent.) large hollow filaments grow out rapidly from the metal within a few seconds. Each such filament advances into the solution at an apparently rapid rate (of a millimeter or more per minute) by a uniform motion; its contour is smooth and regular and its shape cylindrical or slightly tapering; it usually follows a straight course, but is easily diverted by encountering obstacles, such as solid particles or other filaments, or by shaking the watch-glass so as to make irregular the deposition of precipitate at the extremity. In a growing filament this extremity is always open, and from the orifice a stream of liquid containing fine suspended particles flows continually outwards for a short distance in advance, suggesting somewhat the smoke emerging from a chimney; this stream of finely divided material leaves behind and on either side a trail of particles through which the growing tubule advances; while at the edges of the open orifice a dense uniform layer of precipitate is continually being deposited in continuation of that already laid down. Growth proceeds rapidly in this manner until some condition arrests the outflow of fluid; this may happen as a direct result of increasing length, the flow through the long capillary tube being slowed more and more by frictional resistance as the length increases, until finally the end is sealed by the precipitate. Evidently a wide tube will grow longer, under these conditions, than a narrow one, and in fact the great majority of filaments cease growth at a length of one or two millimeters or less; it is only the exceptional wider tubes that reach the length of a centimeter or more.

From these facts it is clear that elongation is dependent upon the maintenance of a flow of solution from the metal along the tube. The precipitation at the open end shows that this solution contains a dissolved salt of the metal. Apparently the process takes place somewhat as follows. At the anodic portions of the metallic surface ferrous ions enter solution; these form with the ferricyanide ions of the solution already present a precipitate of ferrous ferricyanide which is deposited as an incomplete vesicle or ring of semipermeable membrane forming the beginning of the tube; ferrous ions continue to be given off from the iron surface, forming ferrous chloride with the chlorine ions present; the interior of the tube thus contains a solution of ferrous salt which is continually being propelled forward through the open extremity, and there meeting ferricyanide from the external solution forms the precipitate.

The question of what maintains the flow is interesting. One possibility is that each ferrous ion is hydrated, *i. e.*, carries water along with it as it enters solution; water thus flows in the same direction as the ferrous ions, the energy of the flow being derived from the solution-pressure of the iron. Other factors, however, probably enter; it is especially to be noted that the point of origin of each tube represents an anodic area, from which the positive electrical stream enters the solution, reëntering the metal at some cathodic region or regions outside of the tube. An electric current thus passes outward along the tube; and this must cause effects of the kind always found when such a current traverses a system of capillary tubes containing a solution, *e. g.*, a porous partition; these effects are of the kind known as "electrical endosmose";[1] when the substance of the capillary wall is negatively charged the adjacent solution is positive and travels with the positive stream. It is known that iron ferricyanide particles are charged *negatively* in contact with water;[2] hence the layer of solution at the walls of a tube consisting of this material must be *positively* charged and undergo transport in the direction of the positive stream. The flow of solution through the tube is thus probably in large part due to electrical transport by the current of the local circuit. One necessary factor in the process of formation of the tube is the semi-permeability of its wall, which permits water but not salts to penetrate; the solution of ferrous salt thus transported along the tube can form precipitate only where it flows out at the open extremity and meets the ferricyanide outside; part of the precipitate is scattered outside the tube, as already described, or remains finely suspended within its interior; but part adheres at the outlet and contributes to the growth of the tube.

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[1] For a description of this phenomenon see the larger textbooks of physics; a good account of the essential conditions is given in Höber's "Physikalische Chemie der Zelle und der Gewebe," 1914, pp. 234 *seq.*

[2] The ferrocyanides and the ferricyanides of the heavy metals form negatively charged particles. The potential-difference of Prussian blue against water is .056 volt. (*Cf.* Burton, "The Physical Properties of Colloidal Solutions," London, 1916, p. 135.)

According to this conception of the tube-forming process, the rate of flow of fluid along the tube must vary directly with the intensity of the electric current through the tube—*i. e.* (assuming constant E.M.F.), with the electrical conductivity of the solution within the tube. This explains why the rate of growth of the filaments varies directly with the concentration of NaCl (or similar salt) in the ferricyanide solution. The only salt that can remain in solution inside the tube and contribute to the conductivity of its contents must obviously be one that is not precipitated by the ions of the tube-forming metal.

Structure of Filaments.—The structure of a tubule is partly determined by the nature of the material composing it, partly by the conditions under which the material is laid down, such as the size of the anodic area, concentration of salts, presence of protective colloid, external mechanical influences, etc. In general the consistency of the precipitate is coarsest in the case of zinc, and finest in the case of copper; hence tubes of zinc ferricyanide have a coarsely granular aspect and are more irregular in form, while those of iron and copper have smoother contours and a more uniform appearance. There is always considerable variation in the diameters of the tubes formed in any single experiment; in general an increase in the concentration of NaCl in the solution (*i. e.*, more rapid rate of formation) favors the production of wider tubes. Apart from differences in caliber there is little variation in the structure of those tubes which lie entirely below the surface of the solution; typically these are cylindrical or slightly tapering in shape, and follow a more or less tortuous course, varying according to the number and nature of the mechanical obstacles encountered during their formation; zinc filaments show more irregularities of this kind than iron or copper filaments. Those filaments which reach the surface of the solution during their formation undergo various characteristic modifications; thus vesicular or bladder-like thin-walled structures may be formed, especially in the case of zinc, as above described; several such structures derived from different filaments may fuse and form a compound vesicular or cellular mass. One of the most interesting modifications is of a kind often shown by surface-filaments of iron or copper ferricyanide, consisting in a series of transverse striations resembling those of a striated muscle fiber. A regular ladder-like structure of this kind may extend for a long distance; it is apparently due to the presence of alternately denser and thinner zones in the precipitation-membrane forming the wall of the tube. This structure is found only in the larger filaments running along the surface of the solution, and indicates that the deposition of precipitate takes place in an intermittent manner under these conditions; the regular rhythmical recurrence of a metastable condition of some kind, such as supersaturation—as in the phenomenon of the Liesegang rings[1]—may determine its production, but the precise conditions require further study. Occasionally the finely divided precipitate inside the tube may aggregate to form rounded masses repeated at regular intervals, in a manner suggestive of a row of spores.[2] Filaments of zinc ferricyanide may at times exhibit a regularly constricted or moniliform appearance, but they never form regular cross-striations like those shown by iron and copper filaments; the coarser texture of the precipitate is probably responsible for this difference.

Certain other types of precipitation-structure should also be mentioned briefly.[3] Masses of precipitate of a vesicular or chambered rather than filamentous structure are often laid down at the surface of the metal; this is more frequently the case with zinc than with iron or copper. Apparently in such cases the walls of the first formed vesicles remain intact, and do not rupture to allow the solution of metallic salt to flow out in the continuous stream necessary for the formation of filaments. Once formed, these precipitation-vesicles may be displaced or detached by the pressure of others formed later, and large groups or heaps of such structures may thus collect. In the case of copper one highly characteristic type of formation, frequently found in solutions of K_3FeCy_6 containing considerable chloride (*e. g.*, 4 per cent. NaCl *plus* 2 per cent. K_3FeCy_6), is a group of slender tapering filaments grouped radially about a central vesicular mass of precipitate, the whole structure suggesting a miniature chestnut-burr.

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[1] *Cf.* Liesegang, "Beiträge zu einer Kolloidchemie des Lebens," Dresden, 1909. For a more recent study of this phenomenon *cf.* Stansfield, *American Journal of Science*, 1917, Vol. 43, p. 1.

[2] The structure of these tubules resembles closely that of the "osmotic stems" described and pictured by Leduc in his "Mechanism of Life," London, 1911, pp. 141–2.

[3] In Lehmann's "Molekularphysik" various precipitation-formations are compared with different types of organic structure, and much interesting detail is given.

It should be noted that these various types of structure, together with many others, are produced—as is also the case with the majority of living structures—by combinations of microscopic hollow elements, vesicular or tubular in form, with walls consisting of semi-permeable membranes. The above resemblances to organic growths are undoubtedly dependent upon this fundamental similarity of physico-chemical constitution.

Influence of Contact of other Metals upon Filament-formation.—Before proceeding to describe observations showing the inhibiting and reinforcing influence which the contact of one metal may exert upon the formation of filaments from another, the general theory of this effect ought briefly to be considered. As already explained, the formation and growth of these structures depend upon a process of electrolytic local action or electrolysis; the region from which the filament grows out represents an anodic area, *i. e.*, an area where ions of the membrane-forming metal enter solution; these ions then interact with the ferricyanide ions to form the precipitate; to each such anodic area corresponds a cathodic area (or areas) at some other region of the metallic surface, where the positive stream reënters the metal to complete the circuit; at this area hydrogen ions are deionized and hydrogen gas is freed with the formation of alkali. In the case of iron these areas may readily be demonstrated by the addition of a little phenol phthaleïn to the ferricyanide solution (as in the ferroxyl reagent of Walker);[1] the cathodic regions, which typically remain bright and free from filaments for some time, then exhibit the characteristic red color of the indicator in alkaline solution. Each filament thus originates from an anodic area in a local electric circuit; the precise mechanism of the formative process has already been considered.

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[1] *Cf.* Walker, Cederholm and Bent, *Journ. Amer. Chem. Soc.*, 1907, Vol. 29, p. 1251.

Specimens of ordinary iron always exhibit a large number of local couples of this kind, because of the characteristic lack of electro-chemical homogeneity of this metal. As has frequently been pointed out, this condition explains the susceptibility of iron—especially impure iron—to rust; an iron surface is composed of numerous areas which differ in physical condition and hence in electrolytic solution-tension; areas of higher solution-tension form local anodes, and rusting is a consequence of electrolysis at these local circuits. Iron prepared in as high a state of purity as possible is surprisingly resistant to corrosion.[1] What is true of rusting is true also of the formation of precipitates with electrolytes like ferricyanide; filaments form more slowly from chemically pure iron wire than from pieces of ordinary commercial metal (nails, etc.). Zinc and aluminium, although metals of higher solution-tension than iron, are more readily obtained in a relatively homogeneous condition; hence they resist corrosion better and form precipitate more slowly in ferricyanide solutions.

Such considerations explain the accelerating influence which the contact of a nobler metal, like platinum or copper, has upon the formation of precipitation-filaments from zinc, iron, or copper. The nobler metal (that of lower solution-tension) has a higher potential in contact with the solution than the less noble; hence a circuit is formed in which the latter acts as anode, *i. e.*, enters solution as cations which form precipitate with the ferricyanide. Conversely, a less noble metal, *e. g.*, magnesium or manganese in relation to iron or zinc, *inhibits* the formation of filaments from the membrane-forming metal. Both the reinforcing and the inhibiting influences have very pronounced and evident effects, which are perceptible not only at the immediate region of contact of the two metals, but for a considerable distance, *e. g.*, of several centimeters, beyond this region. The degree of this influence at any point on the surface of the filament-forming metal depends in general upon three chief factors; these are, (1) the nature of the second metal, (2) its distance from the point under consideration, and (3) the electrical conductivity of the solution. The electromotive force of the local circuit depends primarily upon the difference between the specific solution-tensions of the two metals; hence under otherwise equal conditions zinc is less effective than manganese, and manganese than magnesium, in inhibiting the formation of filaments from an iron surface. The other two factors, (2) the distance between the contact of the metals and the point in question, and (3) the electrical conductivity of the solution, determine the resistance of the portion of the local circuit which includes that point; this determines the intensity of the current and hence the rate of local electrolysis at the point. The effect is thus greatest near the region of contact and diminishes as the distance from this region increases.[1]

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[1] For references to the literature in this field see my paper above cited, *Amer. Journ. Physiol.*, 1916, Vol. 41, p. 126.

The actual phenomena may now be illustrated by the description of typical experiments. In order to show the accelerating influence of a nobler metal, zinc is more satisfactory than iron, for the reason that it can more readily be obtained in comparatively pure and homogeneous condition, in which state it forms precipitate in ferricyanide solutions very slowly unless in contact with the other metal. It is difficult to procure non-reactive specimens of iron; pure iron wire of the kind used in standardizing (*e. g.*, Baker's labeled 99.7 per cent. pure) forms filaments rapidly in a 2 per cent. solution of K_3FeCy_6 in dilute egg-white; the rate of formation is accelerated by encircling the iron with copper or platinum wire, but the degree of acceleration is comparatively slight, and the effect is much less striking than when zinc is used.

Such experiments as the following are easy to perform and invariably give striking results. A small strip, *e. g.*, 2 cm. long by 1 mm. wide, cut from a bar of pure zinc, is encircled at one end with a ring of fine copper wire, and placed in a 2 per cent. solution of K_3FeCy_6 in dilute egg-white. Within a minute or two the characteristic thin-walled transparent vesicles appear at the surface of the metal, and somewhat later slender filaments begin to grow out; in a short time (half an hour or less) the metal is covered with filaments and vesicular formations from one end to the other. The first appearance of these structures is typically near the contact with the copper; a gradient in the rate of formation is thus apparent, with the most active region near the copper. This accelerating influence of the nobler metal is clearly perceptible at the extremity of the strip, *i. e.*, for a distance of some centimeters from the contact. A similar "control" piece of zinc, without copper, shows only a few irregular and slightly developed filaments at a time when the copper-bound piece is completely covered with a luxuriant hypha-like growth. Platinum and iron wires have the same general effect as copper, only with platinum the accelerating influence is distinctly greater, and with iron less, than with copper. Carbon may also form the substance of the cathodic area; a piece of zinc marked with lead-pencil shows a rapid development of filaments, beginning at the marked area and grading off from there.

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[1] *Cf.* the second paper of my recent series on the physical chemistry of the conduction-process, *Amer. Journ. Physiol.*, 1915, Vol. 37, p. 348.

Striking experiments may also be performed with copper as the filament-forming metal. In this case a higher concentration of NaCl is required for the rapid formation of filaments; beautiful structures develop after some minutes in a solution containing 4 per cent. K_3FeCy_6 and 4 per cent. NaCl (with or without egg-white). A piece of fine copper wire 3 or 4 cm. long, encircled at one end with a platinum wire, or wound around a piece of carbon, shows a highly characteristic development of filaments; some of these within a few hours may reach a length of several centimeters; such filaments, as already described, often exhibit a very perfect and regular cross-striation where they run along the surface of the solution. A similar piece of copper wire without contact of nobler metal or carbon remains unchanged.

Many variants of this type of experiment will readily suggest themselves. Experiments with plated wires yield instructive results. Pieces of "galvanized" (zinc-plated) iron usually show the first development of filaments of zinc ferricyanide near the cut surface where the iron is exposed, and gradients in the rate of formation extending from this region along the wire are frequent. It should be added, however, that these gradients are often obscured by irregularities in the rate of action, due presumably to lack of uniformity in the zinc layer (local interruptions, etc.). Platinum wires coated with copper in an electrolytic bath also make interesting demonstrations; such a wire shows no formation of filaments in a solution of K_3FeCy_6 and NaCl if the coating of copper is continuous; but if it is scraped away at any region so as to expose the underlying platinum, the copper near this region soon puts out filaments, and the process extends for a distance of some centimeters from the area of exposure. Such experiments are instructive as exemplifying the nature of the effects that may follow local alteration in the electromotor character of a surface; and they may be used to illustrate by analogy such physiological phenomena as the initiation of growth-processes or regeneration in living organisms by incision, amputation, or similar treatment.

The reverse type of experiment, in which the formation of filaments from one metal is inhibited or prevented by contact with another metal of higher solution-tension, is equally readily performed. Such an effect is illustrated in the just cited experiment with zinc-plated iron wire; the exposed iron surface develops no filaments; their formation is evidently prevented by the adjacent zinc, which forms with the iron a couple in which the zinc is anode; this, it may be added, is the reason why such wires do not rust. The inhibitory influence of zinc upon the formation of ferricyanide filaments from iron may be shown in a more striking manner as follows; a straight piece of thin bright iron wire (*e. g.*, Baker's C. P.). some centimeters long, one end of which is wound about a small strip of zinc, is placed in a 2 per cent. K_3FeCy_6 solution in dilute egg-white. Filaments put forth rapidly from the zinc, especially near the iron, but the iron itself remains perfectly bright and bare, and may show no development of filaments for hours. If then the wire be cut in two by scissors, the part remaining in connection with the zinc remains unchanged, while the isolated part quickly develops the characteristic blue-green filamentous growth of ferrous ferricyanide. Evidently this development had previously been repressed by the influence of the zinc; when the connection is severed the iron reacts as usual. This experiment is even more striking if a metal of higher solution-tension than zinc is used, such as aluminium, manganese, or magnesium. Magnesium is especially effective, since the ferricyanide of this metal is soluble and hence does not accumulate at the anodal regions and arrest the inhibitory action by polarization; such an iron wire remains bright until the magnesium is completely dissolved; then filaments grow out as usual. Apparently when zinc is used the accumulation of zinc ferricyanide may interrupt contact between the metals or render the resistance of the local circuit too high (*i. e.*, have a polarizing effect), and by degrees filaments form from the iron. In addition to this effect, contact of the zinc ferricyanide precipitate with the iron appears to form local couples in which the iron acts as anode, so that iron in contact locally with this precipitate usually exhibits in course of time a development of blue filaments. This, however, is an incidental effect due to the formation of another type of circuit. Pieces of aluminium or manganese are almost as effective as magnesium in preventing the formation of iron filaments in the above solution. A large variety of experiments of this type may be performed with iron wire. In a similar manner zinc may be used as the filament-forming metal, and the process inhibited by magnesium, aluminium, or manganese.

As already mentioned, this inhibitory influence may be effective for a distance of several centimeters from the region of contact of the two metals, and a gradient in the intensity of the influence is apparent. After the current of the circuit has decreased, as the result of the accumulation of precipitate or other polarization, an iron wire with zinc connection often begins to send out filaments; these tend to be first formed at the region farthest removed from the zinc. Irregularities, however, are frequently observed in such experiments, due no doubt to local irregularities in the composition and physical condition of the iron. The biological analogies to these effects will be considered later.

An interesting type of effect is seen when an iron wire 3 or 4 cm. long is in contact at one end with a piece of zinc and at the other with copper or platinum. With this arrangement gradients in the formation of iron filaments are very distinct; at first these filaments are formed only in the immediate neighborhood of the copper and are entirely absent near the zinc. Copper and zinc influence the reaction of the iron wire with the solution in opposite manners, the former promoting the formation of iron filaments, the latter inhibiting this process. The zinc and the copper, although separated by the length of iron wire, can be shown to modify each other's action; thus filaments grow out from the zinc under the influence of the iron, but more slowly than in the absence of the copper; similarly the growth of filaments from the iron near the copper is much slower than in the absence of the zinc. The effect is as if the formation of filaments from the zinc inhibits or holds in check the formation of filaments from the iron, and *vice versa.* This mutual interference is obviously due to the intersection of the electrical currents from the two circuits, and resulting compensation-effects. Severing the iron wire between the copper and the zinc removes this influence, with the result that then filaments are put forth rapidly from both iron and zinc; *i. e.*, the process at either filament-forming metal is released from a certain constraint, due to the polarizing influence of the electrical current from the other circuit; it then proceeds more rapidly than before. Physiological analogies to this type of effect readily suggest themselves; the development of shoots in one region of a plant stem inhibits their development in adjoining regions; removal of a rapidly growing shoot may thus cause buds to develop from the axils and elsewhere, which otherwise would have remained dormant.[1] Similar phenomena are seen in hydroids and other animals, and will be discussed more fully below. Physiological "dominance" of this type is often simulated in a suggestive manner by experiments of the above kind. For example, a thin strip of zinc 3 cm. long was in contact at one end with a piece of pure iron wire and at the other end with a piece of magnesium. A well-marked gradient was shown; near the iron filaments were rapidly formed from the zinc, but near the magnesium slightly and slowly. It was noteworthy that the piece of iron wire gradually formed filaments in spite of the contact with the zinc; *i. e.*, the presence of the magnesium inhibits the protective influence of the zinc upon the iron,—evidently because the zinc forms the cathode in the zinc-magnesium couple, the current from which opposes and partly compensates that from the zinc-iron couple. In another experiment a similar strip of zinc was in contact with platinum wire at one end and with magnesium at the other; in this case the zinc remained free from filaments for a long time; after 18 hours a dense growth of filaments had developed near the platinum; toward the magnesium the growth decreased, and near the contact with this metal the zinc was entirely free from filaments. In a similar manner the contact of iron or zinc with a platinum-bound copper wire inhibits the formation of filaments from the copper. A piece of copper wire 4 cm. long, encircled at one end with platinum wire, was placed in a solution containing 4 per cent. K_3FeCy_6 and 4 per cent. NaCl; filaments and bur-like tufts of copper ferricyanide appeared rapidly. But a similar piece of platinum-bound copper which was placed in contact at the other end with an iron wire showed only slight and gradual formation of precipitate; under these conditions filaments form rapidly from the *iron*, and their formation apparently has the effect of inhibiting their development from the copper. What really happens is that the current of the copper-iron circuit is opposed in direction to that of the copper-platinum circuit and compensates the latter.

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[1] For numerous examples *cf.* McCallum, "Regeneration in Plants," *Botanical Gazette*, 1905, Vol. 40, pp. 97, 241.

Attention should also be called to a type of experiment in which the two precipitate-forming metals are not in direct contact with each other but are connected by a conductor which itself undergoes no chemical change, like platinum, carbon, or copper. For example, a piece of iron and a piece of zinc connected by a stretch of copper wire 1 cm. long were placed in 2 per cent. K_3FeCy_6 in dilute egg-white; the zinc immediately formed filaments, but not the iron. Similar pieces of iron and zinc, each in contact with a separate piece of copper, both form filaments rapidly, as already described; but when the two are in contact with the same conductor the zinc prevents the formation of filaments from the iron in the same manner (though to a less degree) as when the metals are in direct contact. It is further to be noted that under these conditions the iron, though not itself forming filaments, retards or partly inhibits their development from the zinc; *i. e.*, zinc in contact with copper alone forms filaments more quickly than when in contact with a copper wire which is itself in contact with iron. This mutual influence is greatest when the two metals are close together; when they are separated by a sufficient stretch of conducting wire the effect becomes inappreciable. Thus with a distance of 3 cm. between the zinc and the iron, the latter metal, as well as the zinc, formed filaments, but more slowly than in the absence of the zinc; the influence of the zinc was perceptible, but insufficient to suppress entirely the action at the iron. Increasing the distance of separation still further renders the influence inappreciable. This experiment recalls such physiological phenomena as the decrease of growth-inhibiting dominance with distance.[1] Experiments with platinum as the connecting metal give similar results; only here, because of the nobler character of the platinum, the distance between the zinc and the iron must be less than when copper wire is used, if the formation of filaments from the iron is to be entirely suppressed. In other words, both zinc and iron have a stronger tendency to form filaments when in contact with platinum than when in contact with copper; hence in order to counteract this tendency in the iron its distance from the zinc must be less.

It will be evident that effects of the above kind have many close biological analogies.[2] Increased physiological or metabolic activity at one region of a living organism often results in the decrease or cessation of activity at another, usually adjoining, region. Perhaps the best known instances are seen in various phenomena of growth and regeneration to which attention already has been called; the reciprocal inhibition of antagonistic neurones in the central nervous system is probably a phenomenon of the same essential type. There is also good reason for associating with this class of phenomena such regular and characteristic physiological processes as the automatic initiation and arrest of activity at any region of a conducting element (*e. g.*, nerve fiber) as the advancing wave of excitation reaches and passes that point.[1] In the case of these various vital phenomena it seems highly probable, from the degee and nature of the resemblances which they bear to the simple physico-chemical phenomena described in this section, that the essential determining factors are also electrical currents between adjoining cells or cell-regions. These, according to their direction, local density, and intensity, may initiate, inhibit, or reinforce processes at various points along the path of the current.

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[1] *Cf.* Child, "Senescence and Rejuvenescence" (especially Chapter 9), and "Individuality in Organisms," for a full account of these phenomena (University of Chicago Press, 1915).

[2] See Section III. below, page 171.

It may sometimes be desirable to demonstrate still further the electrical nature of these inhibitory and accelerating influences. This may be done most readily by passing the current from a battery, connected with a rheocord, through the ferricyanide solution, using iron wire as electrodes. The formation of filaments at the anode is promoted and at the cathode is prevented, to a degree which varies with the potential between the electrodes; this potential may be changed at will by shifting the position of the slider.

III. Biological Comparison.

In comparing phenomena of the above described kind with physiological phenomena it is obvious that careful distinction must be made between resemblances that are superficial and resemblances that are based upon a fundamental identity in the nature of the determining conditions. It need scarcely be pointed out that in the specific character of the structure-forming chemical reactions there is no resemblance between the two processes under comparison. The complex and imperfectly understood metabolic transformations in living cells are of a kind entirely different from the simple cross-decompositions and precipitations of the above experiments. The resemblances relate to the conditions under which the chemical reactions take place, to the type of structure formed, and to the mode of transmission of chemical influence from one region to another region which is connected with the first only through the medium of an electric conductor. Chemical action at a distance is a phenomenon universally met with in organisms; and the attempts to explain this action on the basis of transport of actual materials—through the flow of sap, blood, or other circulating medium—are in many cases patently inadequate. Consider—to take an extreme instance—the transmission of chemical influence from the brain to a secreting gland-cell; here obviously the problem of the nature of physiological conduction enters; and it is more especially this problem—the nature of transmission of physiological influence where no direct transmission of material is possible—upon which experiments of the above kind appear to throw light. The problem is plainly a most fundamental one, since physiological coördinations of the most varied nature depend upon the rapid conduction of chemical influence between different parts of the organism. The integration of the whole complex system of structures and chemical reactions into a unified and regularly acting living organism probably depends more essentially upon transmission of this type than upon any other single process.

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[1] *Amer. Journ. Physiol.*, 1916, Vol. 41, see pp. 134–5.

The transmission of formative influences, sometimes of a growth-inhibiting, sometimes of a growth-promoting kind, from one region of the organism to another is a well-known phenomenon in both animals and plants. Usually it has been explained either as due to the production and transport of special materials (organ-forming substances, hormones, etc.), or as due to changes in the distribution of materials already present (changes in the flow of water in plants, increased availability of nutritive substances to one organ when another organ is removed, etc.). In animals a special rôle has at times been assigned to nervous influences; the nervous system[1] or sense-organs[2] have been shown in some cases to influence the rate of regeneration, *i. e.*, a transmissive instead of a transportative means of control[3] has been recognized; but in plants explanations based upon the assumed existence of form-determining substances have been most generally employed (Sachs and others).[1]

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[1] This influence is seen (*e. g.*) in Herbst's experiments in which the regeneration of the eye in Crustacea was found to depend on the presence of the optic ganglion; also in planarians and other forms.

[2] The presence of the marginal sense organs in regenerating pieces of the medusa *Cassiopea* favors regeneration, according to the recent observations of Cary, *Journ. Exper. Zool.*, 1916, Vol. 21, p. 1.

[3] For a discussion of the general nature of this influence see Child's "Individuality in Organisms," especially Chapter 6.

Among the various phenomena of organic growth and development, regenerative processes seem most evidently open to interpretation in the light of the foregoing experiments. In the regenerating organism growth or development is initiated as a result of the removal of one of its parts, the usual result being the restoration of the missing part or its equivalent. In many cases the part removed is replaced by growth at the cut surface; in other cases the remaining portion of the organism undergoes a more or less extensive reorganization, called by Morgan "morphallaxis"; in still others, especially in plants, removal of an organ initiates the development of a similar organ from another locality, *e. g.*, when the removal of the growing tip induces the development of lateral branches from axillæ or other regions at a distance.

The first class of cases, where growth at the cut surface restores the missing part, may be compared with the case of a membrane-forming metal in a ferricyanide solution, *e. g.*, a piece of iron wire in which the filamentous or membranous growth has accumulated to a degree sufficient to insulate the metallic surface from the surrounding solution, *i. e.*, to arrest the flow of current between anodic and cathodic regions by the interposition of the non-conducting coating of precipitate. If then a cut be made exposing a fresh surface of metal, the formation of precipitate is at once resumed and continues until a new state of equilibrium is established; the accumulation of electrolytically formed structure will then be approximately the same as before. The whole structural system, consisting of the metal with its outer coating of precipitate, will exhibit essentially the same constitution as before. Again in the case of a strip of zinc encircled by a copper wire, as in the experiments described above, the zinc forms the anode of the couple, and its surface is soon covered by precipitation-structures, whose accumulation progressively retards the flow of current and with it the structure-forming electrolysis. When the fresh surface of zinc is exposed, the formation of a new filamentous coating follows simply from the reëstablishment of the electrical circuit. As the current flows, structural material is formed and deposited at the anode, until its accumulation increases the resistance of the circuit and checks the current and hence the electrolysis which produces the structure. Similarly in the case of a regeneration-process where proliferation starts at a cut surface at which previously quiescent cells have been exposed, it is possible that the condition of quiescence is due to insulation by the surrounding cells and other tissue-elements; if these are removed the flow of an electrical current and the formation of new structure are enabled to take place.

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[1] For an experimental examination and critique of this type of hypothesis, *cf.* the paper of McCallum above cited.

The case of morphallaxis is more complex, and is difficult to simulate by a model of the above kind. In this case constructive processes at one area are accompanied by regressive processes at another area; *i. e.*, growth at one region forms the condition of inhibition of growth or of positive regression at other regions; the whole mass of organized living material is thus worked over or reconstituted until a second equilibrium is reached; the character and distribution of structural material is then similar to that existing before mutilation took place. The conditioning of inhibitory processes at one region by active processes at another, and *vice versa*, is a frequent phenomenon in organisms; and analogies are afforded by the experiments cited in the preceding section. In general, in any electrolytic couple the processes at the anode are of an oxidative, those at the cathode of a reducing nature; and it is not difficult to understand how in a living organism increased chemical activity of a certain kind at one region (*e. g.*, oxidation) should result in decreased activity (or activity of the reverse type) at another region related to the first in the manner in which the electrodes of a circuit are related to each other. The inhibition of filament-formation at iron surfaces by the contact of a baser metal illustrates an essentially similar condition. The constant form-relations of an organism or of its parts may thus represent a condition where a complex system or combination of electrolytic processes, interrelated as above, mutually equilibrate one another, such equilibration involving a certain constancy in the relative positions, dimensions, and form-characters of the chief structural elements. This condition would imply a constancy in both the morphological and the physiological constitution of the system, which would be regained after disturbance. I introduce this abstract conception of the organic equilibrium in order to suggest a general point of view from which the actual phenomena of form-regulation may perhaps be considered to advantage. There is much evidence indicating that many structural correlations are controlled by electrolytic processes of the above kind.

Morphallaxis is essentially a phenomenon of form-correlation, which is exemplified by the third type of process mentioned above, in which the growth of one part may be promoted or inhibited by growth in another part more or less distant from the first. For example, in plants the development of new buds or roots is prevented so long as the main growing tip or root-system is intact; formative inhibitions of this type are extremely frequent; hence the isolation of an organ, by removing the inhibiting influence of other parts, may cause the development of roots, shoots, or other organs of a kind entirely different from itself, The whole organism may thus be reproduced in certain cases; the leaves of *Begonia* and *Bryophyllum* furnish the most striking instances of this phenomenon. In plants the normal correlation between the growth of different organs appears to depend largely on this influence. In the lower animals various regions or parts of the organism are also capable of reconstituting the whole when removed from the influence of other regions. In many axiate animals the more anterior regions thus "dominate" the more posterior regions, and a gradient of influence exists, as shown in detail by Child in his recent remarkable book.[1] The controlling influence is greatest near the dominant region and decreases with increasing distance, so that a sufficient degree of isolation for independent development may often result from simple increase in length; *e. g.*, in a planarian or a hydroid stem the portion of the animal most distant from the head end or the hydranth may form a new individual while still connected with the parent. According to Child, individuation in either asexual or sexual reproduction consists essentially in the removal of one region of the organism from the growth-inhibiting influence of other regions; the region thus physiologically isolated then develops on its own account. Cases of compensatory regulation,[1] *i. e.*, where the rate of regeneration is greater the greater the number of the missing parts, may be understood as due to the more complete removal of the inhibiting influence exerted normally by those parts. In other cases one region may promote or reinforce the growth or development of another region; the nervous system appears to have this relation to regeneration in a number of cases; the presence of sense-organs promotes regeneration in Cassiopæa; the intestinal tract has a similar influence in planarians.[2]

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[1] "Senescence and Rejuvenescence," especially Chapter 9. See also the same author's "Individuality in Organisms."

Some of the more suggestive parallels between regenerative or growth processes and the behavior of combinations of filament-forming metals have already been pointed out. The formation of precipitation-filaments from one metal is inhibited by the contact of another metal of higher solution-tension. Hence an iron wire connected with a piece of manganese, aluminium, zinc, or magnesium remains free from filaments for a considerable distance from the region of contact. If a piece of such wire be isolated by cutting, it immediately develops filaments. Similarly iron wire connected with one of these metals through a short copper wire develops no filaments; cutting the connecting wire immediately initiates their formation. These instances are sufficient for illustration of this kind of effect. The precise results obtained vary with the special nature of the conditions, and may be controlled at will by the experimenter.

The reverse class of cases where the formation of filaments is promoted, instead of inhibited, may be illustrated by a strip of zinc in contact at one end with a piece of carbon, platinum, copper or iron. Filaments are formed from all parts of the zinc surface, most rapidly near the region of contact. If then part of the zinc is severed from its connection with the other metal the development of filaments from the isolated part is at once checked; similarly with the whole strip if the platinum or copper is removed. One might compare the influence of the latter metal with that of the sense-organs in a regenerating portion of a medusa. The influence of the nucleus in cell-regeneration and cell-growth is another possible parallel of a more general kind,

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[1] *Cf.*, *e. g.*, Zeleny's work on brittle stars and other forms, *Jour. Exper. Zoöl.*, 1904, Vol. 2, p. 1.

[2] Instances of the influence of the nervous system and sense-organs have been already cited; for the case of the intestinal tract, *cf.* Bardeen, *Amer. Journ. Physiol.*, 1901, Vol. 5, p. 1.

Cases of reciprocal influence, where two or more different organs exercise mutual control upon one another's growth or development are also frequent in organisms. The development of roots and shoots from the leaves and stem of *Bryophyllum*, as recently described by Loeb,[1] furnishes very clear illustrations of this phenomenon; the growth of a bud on a piece of stem inhibits the growth of roots on an attached leaf; if the bud is removed, roots grow out; similarly roots growing from the stem inhibit the formation of shoots from a leaf. Such cases resemble the above combination where zinc in contact with a copper wire inhibits the development of filaments from iron also in contact with the wire; this influence is also reciprocal, although zinc, because of its greater solution-tension, has the predominant effect.[2] In the inorganic model the direction and intensity of the electrical current flowing between the precipitate-forming metal and the solution, and hence the rate and the character of the structural development, depend upon the special characteristics of the two intersecting circuits. The conditions in the living organism are probably similar in a general sense; and compensations and reinforcements occur, varying according to the character of the connections, the distances and other space-relations, the electrical resistances, etc. Other factors, however, including probably the flow of dissolved materials, almost certainly enter in actual cases of growth and regeneration in organisms. Nevertheless it appears probable that the most general and fundamental basis of a reciprocality of this kind is electrical; morphological polarity, a closely related phenomenon, would then have a similar basis,[1] and probably also other cases of reciprocality, like reciprocal inhibition in the nervous system. It is well to recognize, however, that together with this factor others often undoubtedly enter, partly independent, partly dependent upon the electrical factor.[2]

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[1] J. Loeb, *Botanical Gazette*, 1915, Vol. 60, p. 249; 1916, Vol. 62, p. 293; 1917, Vol. 63, p. 25.

[2] Loeb enunciates the rule: "If an organ *a* inhibits the regeneration or growth in an organ *b*, the organ *b* often accelerates and favors the regeneration in *a*." (*loc. cit.*, 1915, p. 276). This kind of relationship is also exemplified by the mutual influence of zinc (= *a*) and iron (= *b*) in direct contact with each other. Other well-known general facts of regeneration may be simulated by arrangements of the above kind. Thus the development of a polyp on a tubularian stem inhibits the development of polyps at other regions of the same stem, just as the outgrowth of a shoot on a plant stem inhibits growths from neighboring axils, etc. Similarly the formation of precipitation-filaments from a piece of zinc in contact with a copper wire is checked by the contact of another piece of zinc (or iron, etc.) with the same wire at not too great a distance.

The distances through which these inhibitory and accelerating influences are perceptible in metals vary with the conditions, the chief factors being the nature of the metals in contact and the electrical conductivity of the solution. An iron wire in contact with a metal of higher solution-tension (Al, Mg, Mn, Zn), and immersed in a 2 per cent. albumin-containing solution of K_3FeCy_6, remains free from filaments for a distance of several centimeters from the region of contact. Conversely, a strip of zinc in contact with a nobler metal (Pt, Cu, Fe) or carbon shows an accelerated formation of filaments for a similar distance. Theoretically, the current of the circuit extends through the whole of the metal and the adjoining solution; the intensity of the current between metal and solution at any point is a measure of the rate of electrolysis at that point (Faraday's law); this intensity is determined by the E.M.F. of the circuit and by the resistance of the intervening stretch of solution (that of the metal being negligible in comparison). The rate of action is thus greatest near the metallic junction, and grades off from there. A similar rule will hold for the formative or other influence of a circuit arising through local alteration in a living cell, tissue, or organism. Any local alteration of potential, due to injury, increased metabolic activity, or other condition, may thus exert an electrical influence at a distance from the site of alteration. In the case of a single cell, or other continuous element like a nerve, a current typically flows through the extracellular part of the circuit *toward* the region of alteration or physiological activity; presumably such a current produces at different points along its course the usual physiological effects of electrical currents, varying according to its intensity, local density, time-relations, etc. It is to be presumed that in a similar manner an actively growing region has a different potential from a quiescent region, and that the circuit which includes this active region influences processes in the tissue at a distance. Mathews found the regenerating end of a Tubularia stem *negative* relatively to other regions (in the same sense in which an active or injured region of, *e. g.*, a nerve fiber is negative; *i. e.*, within the living element the positive stream flows away from the active region); and he points out the possibility that the current thus traversing the tissue may influence metabolic and other physiological processes at different regions along its course, and thus may be an important factor in the initiation and coördination of the formative or other activities concerned in regeneration.[1] With this general conception I am in full agreement. The experiments described above show in how sensitive a manner the rate of formation of precipitation structures responds to variations in the intensity and direction of the local electrical currents traversing the system; and the structural and other conditions in living systems are sufficiently like those in the artificial models to warrant the belief that electrical currents must influence in a similar manner the physiological formative processes. The closeness of many of the above parallels between the behavior of the two classes of systems confirms this belief.

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[1] *Cf.* Mathews, *Amer. Journ. Physiol.*, 1903, Vol. 8, p. 294.

[2] *E. g.*, the flow of a hormone-containing solution may depend on an electrical endosmose effect, etc.

It should be emphasized that the mode of transmission which prevails in processes of growth and development appears to be fundamentally of the same nature as the mode of transmission of the excitation-state in irritable and conducting tissues like muscle and nerve. I have dealt with the special features of this latter mode of transmission in a recent series of papers in the *American Journal of Physiology*.[2] In this case the bioelectric circuit between the active and the inactive regions of the irritable element appears to be responsible for the transmission of the physiological effect; the current traversing the adjoining resting region initiates there the same state of activity as that originally excited at the region of stimulation; the region which thus secondarily becomes active repeats the effect at regions beyond, and so the impulse spreads. There need be no decrement in this type of transmission, since an effect of the same kind and degree is automatically reduplicated at each point reached by the excitation-wave as it passes along the tissue.[1] In the transmission of the influence of a growing or regenerating or otherwise physiologically dominant region this reduplication is apparently not present, and hence a gradient of influence or "decrement" is shown. Otherwise the conditions are to be regarded as essentially the same in both types of transmission. It is possible, however, that combinations of the two may occur, giving rise to intermediate conditions.

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[1] *Cf.* Mathews, *loc. cit.*, p. 299. The following quotation from Pfeffer's "Physiology of Plants" is also apposite here: "Weak electric currents continually circulate in plants"; these are "partly maintained by chemical and physical agencies at work in the plant itself. It is possible that these currents may influence metabolism," etc. (English translation, Vol. 2, p. 106.)

[2] *Amer. Journ. Physiol.*, 1914, Vol. 34, p. 414; 1915, Vol. 37, p. 348; 1916, Vol. 41, p. 126.

Transmission of formative influence in development or growth, and transmission of the nervous type, are thus seen, according to the present theory, to be simply different manifestations of the same fundamental type of phenomenon, namely the transmission of an electrical influence associated with a flow of current around the bioelectric circuit. The physiological effects depend upon this current. Such a theory unifies a large number of apparently diverse phenomena. Thus the connection between growth processes and the assumed flow of current becomes intelligible if processes of electrolysis are in fact concerned in organic growth, as well as in the growth of the artificial formations described above. It also accounts for the rapidity of the nervous type of transmission in its most highly developed form, as pointed out in my earlier papers just cited. The transmission of the electrical influence through a circuit is instantaneous, whether the conductor be electrolytic or metallic.[1] A local change of potential in a stretch of nerve thus produces instantly an electrical change at all points of the circuit constituted by the nerve and its medium, including therefore inactive regions of the tissue at a distance from the immediate site of alteration; and if the effect at any such region be sufficiently intense, a second state of excitation is there aroused, and a similar effect is transmitted from this second region to other resting regions beyond. As a striking concrete illustration of the instantaneous transmission of electrolytic effects along a conductor to a distance from the region immediately affected, I recommend the following simple experiment. A straight piece of copper or platinum wire, *e. g.*, 20 cm. long, is immersed in a vessel containing dilute H_2SO_4; the wire remains unaltered. If then one end is touched with a piece of zinc, instantly bubbles of hydrogen start out from the surface of the wire along its entire length. The transmission of the effects of a local stimulus in such a tissue as nerve is conditioned by a process of the same type, according to the present theory; hence it is independent of the transfer of material; and the velocity with which the impulse is propagated is limited only by the degree of sensitivity of the tissue to slight electrical disturbance and by the rapidity with which the local response takes place.[2]

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[1] Child assumes the existence of a decrement in the nervous type of transmission, similar to that observed in the transmission of formative influence; but this assumption seems to be unnecessary. The existence of trapped excitation-waves in rings of tissue, lasting in some cases for days—*e. g.*, in the medusa *Cassiopea* according to Mayer (*cf. Amer. Journ. Physiol.*, 1916, Vol. 39, p. 378)—proves that the impulse is renewed or recreated with undiminished intensity at each region reached by it in its course. In such a case there is obviously no decrement. It is now well established that the "all-or-none" law holds for nerve; this fact in itself would be incompatible with the existence of a decrement, since it implies that each region responds with the same degree of intensity, irrespective of the strength of the stimulus. The existence of a decrement in nerve during fatigue, anæsthesia, or asphyxia does not prove its existence under normal conditions.

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[1] *I. e.*, the speed is that of transmission through the ether—equal to the velocity of light, or of electricity through a metallic conductor (apart from retardation due to electrostatic influences or self-induction). An experimental investigation of the velocity of the electric current through solutions is described in the book by Monroe Hopkins, "Experimental Electrochemistry" (London, 1905). The author reaches the conclusion: "An electrolyte solution conducts the electric current as rapidly as a conductor of the first class, regardless of its composition, provided we have an equal ohmic resistance of a non-inductive type" (p. 76).

[2] In a recent article (*Amer. Journ. Physiol.*, 1917, Vol. 42, p. 469) Mayer has objected to this theory of transmission (which I have called the "local action theory") on the ground that it is "too simple." Nothing, however, but the speed of transmission of the excitation-wave, and the identity of its effects with electrical effects (stimulation, inhibition, etc.), is directly explained by the theory in question. The *entire* phenomenon of nerve conduction is indeed exceedingly complex, including metabolic and other factors of a nature as yet imperfectly known; but the detailed nature of these factors need not be regarded by this general theory, which aims merely at accounting for the possibility and rate of nervous and other forms of physiological transmission where direct transfer of material does not enter. This it does by referring the phenomenon to a more general class, regarding which our

Further light appears to be thrown upon the connection between bioelectric currents and formative processes by the above described phenomenon of rhythmical cilia-like vibratory movements of precipitation filaments. This phenomenon is frequent in newly formed filaments from iron and copper. The rhythm is typically slow, varying from one to five per second, and the movements are found only in the smaller filaments during the earliest stages of their formation, usually ceasing within a minute or two after their first appearance; at times, however, they may last for five minutes or more, especially with copper filaments. It appears probable that the phenomenon depends upon a rhythmical intermittency in the outflow of the solution contained within the tubular filament. As shown above, the metallic area at the base of the filament is an anode, the corresponding cathode being situated outside. The flow of solution through the tube is continuous so long as the extremity remains open, and is accompanied by an electric current. If, however, the opening be temporarily sealed by the deposition of precipitate, both the liquid flow and the electric current must at once cease; such a cessation of the liquid flow probably has a certain mechanical effect upon the position of the filament, while the electrical variation causes a change of surface-tension; and it seems likely that both effects combine to cause a movement, which has the effect of reopening the extremity of the filament and renewing the former condition. A rhythm is thus started which may continue for some time with often remarkable regularity. The determining condition of the phenomenon would then be the alternate disruption and reformation of a part of the tubular membrane, with corresponding opening and closing of the electrical circuit through the tube; the mechanical effects would result partly from the interruption of the liquid flow, but probably chiefly from the electrocapillary variation. Both the energy and the material for the process are furnished by the electric current through the tube.

knowledge is in many respects exact. If the transmission of the local excitation-state is due to the formation of local electrical circuits having the properties of electrical circuits in general, the difficulty of accounting for the rapid propagation of the excitation-wave along a highly irritable and rapidly responding element like a nerve-fibre at once disappears.

The comparison with ciliary movement may with advantage be pursued somewhat further. In the living structure the continuance of the vibratory movement has been shown in many instances to be dependent upon the presence of the so-called basal particle (or "blepharoplast"), which is also the center out of which the structure grows in its development; severance of the ciliary filament from its base instantly arrests the movement.[1] A connection between the conditions of contractile activity and those of growth is thus shown; both processes depend upon changes taking place in the basal granule, which represents a specially modified portion of the protoplasmic surface layer, differing in chemical composition from other regions of the cell-surface (as shown by its staining reactions), and presumably also in electrical potential. This connection becomes intelligible if we assume that (just as in the above inorganic model) the basal particle represents an area from which a supply of formative and energy-yielding material flows out along the filament to replace the material which is altered or consumed at each ciliary stroke. This flow of material is to be regarded as associated with an electrical current, which being interrupted intermittently causes intermittent polarization-effects, with resultant variations of surface-tension producing the contractions. It is clear that a constant renewal of substance is necessary to maintain the integrity of the cilium and to furnish the energy of its contractions; and the resemblance between these structures and the artificial filaments described above appears to be too close not to be based upon some fundamental identity in the conditions of both their origin and their activity. The wide distribution of these vibratile cellular outgrowths, ranging from the lowest unicellular organisms to the highest animals, as well as the readiness with which they are formed at the free surfaces of cells, indicates that some general and simple condition determines their formation, and also that the conditions of their formation and of their contractile activity are closely connected.[2]

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[1] *Cf.* Peter, *Anatomischer Anzeiger*, 1899, Vol. 15, p. 271; Verworn, *Pflüger's Archiv*, 1890, Vol. 48, p. 149.

[2] The fact that bioelectric variations in other active tissues (nerve, muscle) are so frequently rhythmical in character is significant in relation to the problem of the conditions of ciliary movement. It is possible that a rhythm or intermittency

Another remarkable feature of these precipitation-tubules, already described, is that their formation is associated with a centrifugal flow of fluid along the tubule. This is not surprising when it is considered that they originate by a process of electrolysis at local electrical circuits. The passage of an electrical current through a porous diaphragm or other system of capillary tubes has long been known to involve a flow of the solution through the tubes; this is the phenomenon of fluid-transport by the current known as electrical endosmose. The possibility that this process plays a part in the transport of fluid through cells, *e. g.*, in secretion, has at times been touched upon by physiologists;[1] but in the absence of positive evidence it has been for the most part disregarded. Nevertheless it seems certain that currents of fluid must accompany the passage of electrical currents through or between cells, and also that the flow must be in general in the direction of the *positive* stream. Living protoplasm is a polyphasic system, largely fluid in its consistency, and pervaded by solid structural elements (membranes, fibrils, etc.) composed of colloidal material (protein, lipoid) which is charged negatively in contact with neutral or slightly alkaline media, such as those forming the tissue-fluids and the fluid part of protoplasm. The fluid layers in contact with such structures will accordingly be *positively* charged; and displacement of this fluid in the direction of the positive stream is inevitable if the protoplasm forms part of an electrical circuit. Protoplasm always contains salts and is a good conductor of electricity, and its enclosing membranes are readily permeable to water; hence the conditions for such a flow are present. Accordingly we should expect the passage of currents, bioelectric and other, through cells to be associated with a flow of fluid. Apart from such *a priori* considerations, there is ample evidence

in the state of electrical surface-polarization is general in living cells, and that the activity of cilia—contractile prolongations of the cell-surface—is one index of this condition. Any free cell-surface having intermittent polarization might thus give rise to cilia.

[1] *Cf.* Engelmann, *Pflüger's Archiv*, 1872, Vol. 6, p. 97; Waymouth Reid, *Philosophical Transactions*, 1900, Ser. B, Vol. 192, p. 239; Höber, *Pflüger's Archiv*, 1904, Vol. 101, p. 607. Experiments showing transport of water through tissues (muscle and nerve) by the constant current in the direction of the positive stream are described by Hermann, *Pflüger's Archiv*, 1897, Vol. 67, p. 240.

in many physiological processes of a movement of fluid through cells, or from one region to another within cells, *e. g.*, the activities of gland-cells—which are normally associated with bioelectric currents flowing in the cells toward the secreting surface[1]—and the protoplasmic flowing movements in plant-cells. Certain recent observations of Chambers[2] on the micro-dissection of sea-urchin eggs illustrate the readiness with which flowing movements may be set up in protoplasm by local injury of the cell-surface, *i. e.*, by conditions which are known in general to render the cell-surface locally negative. The application of a needle to a sea-urchin egg in a hanging drop "produces peculiar currents in the egg-substance. The currents pass directly from the pushing object in a straight line through the egg to the anterior end where they curve outward and flow back along the surface to be caught again in the flow from the pushing object." The region of contact is presumably negative relatively to other regions of the cell-surface, *i. e.*, the positive stream flows outside the cell from the unaltered to the altered part of the surface and thence through the cell; the observed flow of fluid is thus in the direction of the positive stream. The possibility that dissolved materials may be thus transported from one region of the cell to another ought especially to be noted. If such a condition is general, a flow of fluid in reference to any temporarily altered region of the cell-surface will take place as a result of electrical transport; and this flow may largely determine the supply of material for the repair or reconstitution of the altered area. The fact that in the microscopic precipitation-tubules described in this paper a flow of fluid, due apparently to electrical transport, actually plays an essential part in the structure-forming process, is highly suggestive in relation to the problem of the conditions of structure-formation in cells. There is also evidence that a centripetal flow of fluid from the polar and extra-equatorial regions of the cell-surface takes place in eggs and other cells during mitotic division, and that the astral rays are in part an expression of this flow, the central "sphere" representing an accumulation of the more fluid part of the protoplasm. Chambers brings evidence from micro-dissection favoring the view that the sphere is formed by the confluence of a system of radiating channels containing a more fluid "hyaloplasm" separated by radiating protoplasmic tracts of a solid or gel-like consistency. If the general surface of the dividing cell outside the equatorial area is electrically negative (in the usual physiological sense) relatively to this latter area, as the character of the form-change and certain other facts indicate,[1] then the positive stream in the cell-circuit must enter the surface at the polar and circumpolar areas and leave at the equatorial area. The conditions for a centripetal flow of fluid from the polar areas, due to electrical endosmose, would thus be furnished. The relation of such a flow to the general form-characters of the mitotic figure should be reconsidered in the light of these possibilities.

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[1] *Cf.* Langley, Schäfer's "Textbook of Physiology," Vol. 1, p. 517. Unfortunately this observation does not in itself indicate whether the electric current is the *cause* or *result* of the movement of fluid through the gland. A flow of solution through a porous partition, however caused, gives rise to an electric current.

[2] Chambers, "Microdissection Studies," *Amer. Journ. Physiol.*, 1917, Vol. 43, p. 1; *cf.* p. 7; also *Journal of Exper. Zoöl.*, 1917.

IV. The Possibility of Electrolysis at the Semipermeable Cell-boundaries.

Throughout the foregoing comparison between electrolytic precipitation-growths and organic growths it has been assumed that the formation of electrical circuits between different regions of the cell-surface, and associated with processes of the nature of electrolysis, is possible; and that the conditions under which such circuits arise are of the same essential nature as those determining the formation of local circuits between different parts of a metallic surface. This assumption may not appear at first sight to be consistent with our knowledge of the general conditions under which circuits are formed by chemical action, and electrolysis takes place. In the kind of process considered as typical by electrochemists, a part of the circuit always consists of a metallic conductor; this connects the two ion-forming or ion-combining surfaces (electrodes) which are in contact with the electrolyte-solution. In the living cell it is obvious that no conductor of the first class is present. In other respects, however, the cell-surface resembles the surface of a metallic electrode; local alteration—injury, stimulation, chemical change—sets up local differences of potential, and currents flow between altered and unaltered regions. The potentials thus arising may be summed, polarizability is marked, and the cell-surface is water-insoluble—*i. e.*, there is a sharply defined boundary between protoplasm and surrounding medium. The chief difference in the conditions is that the conduction of electricity both inside and outside of the cell in a bioelectric circuit takes place entirely by means of conductors of the second class, *i. e.*, by ionic movement. Both protoplasm and medium are as a rule good electrolytic conductors; and the surface of separation consists of a thin semi-permeable partition composed of chemically alterable material which is of course non-metallic in character. Can true electrical circuits, in which chemical decompositions of the nature of electrolysis take place at the interfaces, originate under conditions of his kind? This question must be considered before the above comparison can be regarded as a valid one and acceptable conclusions be based upon it.

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[1] *Cf.* R. S. Lillie, *Journ. Exper. Zoöl.*, 1916, Vol. 21, p. 369.

First of all it is necessary to guard against arbitrary preconceptions of the necessary character and arrangement of the different components present in an electrical circuit depending on chemical action, such as we are familiar with in the different types of battery. In all batteries there are at least two contiguous conducting media, the one metallic, the other consisting of an electrolyte-solution; at the boundary-surface between metal and electrolyte chemical changes takes place which furnish the current; the energy of the latter is thus transformed chemical energy. In the usual diagrammatic representation of these conditions, the terminals of the one conducting medium, the metal, are regarded as immersed in the other, *i. e.*, the electrolyte; these terminals, where electricity passes between metal and solution, are the *electrodes*, while the intervening stretch of metal is usually conceived as a wire passing through the air. This last, however, is obviously an arbitrary laboratory arrangement; it facilitates consideration of the processes at either electrode to have each isolated from the other, and the connecting wire removed from the possibility of chemical change; the regions where the essential reactions occur are thus spatially distinct and the processes can be investigated separately. But evidently immersion of the conducting wire in the solution does not alter the conditions in any essential way. A piece of metal completely immersed in an electrolyte-solution, and of different composition or solution-tension at any two regions, or in contact at two areas with different solutions, is equally the seat of chemical change which is associated with the passage of an electric current between the two regions. This is the arrangement which corresponds more obviously to the conditions usually met with in nature. The case of local rusting in iron, or of the transmission of chemical influence (*e. g.*, excitation) from one region of a cell to another, exemplifies such an arrangement. It is important not to allow our conceptions of natural processes to be limited by the peculiarities of laboratory devices; these are always more or less arbitrary, and designed not only for convenience and reduplication at will, but with the special purpose of isolating the phenomena and making them as sharply defined as possible. In unmodified nature such conditions are rarely found; hence such resemblances as that between local chemical action in metals and the physiological effects following local alteration in living cells are not easily recognized.

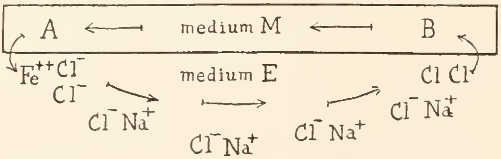

FIG. 1. In this diagram medium *M* represents the metallic part of the circuit, *e. g.*, a platinum wire, medium *E* the adjoining electrolyte solution, *e. g.*, NaCl. Ferrous chloride is in contact with the wire at one end (*A*), chlorine at the other (*B*). The arrows show the direction of the electrical current (positive stream) in the circuit.

A simple type of oxidation and reduction cell seems best adapted to illustrate the essential nature of the phenomena under consideration, especially since the energy of vital processes, including that of the bioelectric currents, is usually derived from oxidations. Probably the simplest case is that of an easily oxidizable salt, *e. g.*, ferrous chloride, in contact with a chemically indifferent conductor, *e. g.*, carbon or platinum, which is immersed in a solution of an electrolyte, *e. g.*, NaCl. The ferrous salt may be in contact with only one region of the platinum wire, *e. g.*, one of its extremities; if now an oxidizer (*e. g.*, chlorine, bromine, nitric acid, etc.) be brought in contact with the other end of the wire, the ferrous salt is at once oxidized to ferric. The subjoined diagram (Fig. 1) will illustrate, in which chlorine is regarded as the oxidizer. The rate of the process depends upon the concentrations of the $FeCl_2$ and Cl_2 at the electrode regions, the area of the electrodes, and the electrical conductivity of the circuit. Each atom of chlorine reaching the wire at region B causes instant oxidation of a molecule of $FeCl_2$ to $FeCl_3$; *i. e.*, each ferrous ion (Fe^{++}) becomes a ferric ion (Fe^{+++}) by receipt of a positive charge from the wire; this charge comes from the chlorine atom which releases it to the wire, becoming at the same time a chlorine ion (Cl^-). The number of chlorine ions in solution thus necessarily increases at the same rate as the number of ferric ions, and the only evident chemical changes are those occurring at the contact of the oxidizing and reducing substances with the wire. The current (positive stream) flows in the solution from the iron salt to the chlorine, and a certain process of rearrangement or change of ionic partners may be conceived as taking place throughout the whole intervening stretch of electrolyte-solution.[1]

The above account will indicate sufficiently the general character of the physical and chemical changes taking place in any oxidation and reduction cell. At the anodic area Fe^{++} ions are oxidized to Fe^{+++} ions; at the cathodic area electrically neutral chlorine atoms are reduced to Cl^- ions. The wire serves simply to convey electricity from the one region to the other. Similarly any oxidizer which is capable of being reduced by $FeCl_2$ may be substituted for chlorine; and any substance which is capable of being oxidized by the oxidizer may be substituted for the ferrous salt.[1] The above substances serve merely to furnish the simplest possible illustration. Oxidation at the anode, reduction at the cathode, with accompanying flow of current between the two regions, form the constant features of the phenomenon; obviously the energy of the current is derived from the energy of the oxidation. It will thus be readily understood that the chemical changes at the electrodes may in some cases be much more complex than those cited above for illustration; and that under certain conditions they may result in decompositions and syntheses of a far-reaching character.

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[1] It is impossible to give a full exposition in this place, and the reader is referred to the textbooks of electrochemistry (Arrhenius, LeBlanc, Lüpke, etc.) for a more detailed treatment. The electron theory includes all of the above phenomena as special cases. From the standpoint of this theory it is more correct to regard each Fe^{++} ion as yielding an electron (negative charge) to the wire at region *A*; this displaces a corresponding electron at *B*; this electron is taken up by the Cl atom which thus becomes a Cl^- ion. The flow of electrons constitutes the current in the wire; at the electrodes there is equal and simultaneous loss and gain of electrons by the substances (ions or other dissolved substances) at anode and cathode respectively.

In the above diagram one half of the circuit, designated as medium *M*, is regarded as consisting of a metallic conductor which itself undergoes no chemical change; the other half, medium *E*, is the electrolyte solution. Obviously a metallic conductor which is not chemically indifferent but is capable of combining chemically with the ions of the electrolyte-solution will produce similar electrical effects. A metal of low solution-tension like zinc or iron will contribute cations to the solution at the anode; these will form chemical combinations according to the nature of the substances present in the solution. Or the reducing influence at the cathode will affect all reducible substances there present; if, *e. g.*, the cathode is composed of a conducting oxide like copper oxide, as in the Edison cell, this is reduced to the metallic state. Evidently the more complex the material of anode and cathode, and the more complex the contiguous solution, the more numerous the chemical transformations that may occur. In all cases, however, Faraday's law holds, and the energy freed in the resulting process is strictly dependent upon the difference in the chemical potential of the substances undergoing transformation at the two regions.

The case does not appear to be altered in essential principle when medium *M* is considered to be not a metal, but a second electrolyte-solution, separated from medium E by a thin partition which prevents diffusion (*i. e.*, is semi-permeable) and can undergo the chemical changes necessary for the generation of an electric current, *e. g.*, oxidation at one region simultaneously with reduction at another. Under these conditions medium M may be permanently of a different composition from medium E, both are electrical conductors of the electrolytic kind, and local chemical changes resulting in ionization or deionization within the substance of the partition-film will result in corresponding electrical effects which may be transmitted and produce chemical effects at a distance just as in the case considered above. This appears to be the type of arrangement exemplified in the living cell.

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[1] See the text-books of electrochemistry for examples of the various possible combinations. An oxidizable organic substance, *e. g.*, pyrogallol, α-naphthol, sugar, may be the substance at the anode; a peroxide the oxidizer at the cathode. These examples are adduced to suggest biological possibilities.

To understand how such currents may be formed without a metallic conductor it is necessary to consider more closely the nature of the part played by this conductor in the usual type of circuit. In the above example the platinum wire serves simply as a means for transmitting electricity, a positive charge passing to (or a negative away from) the metal from each Cl atom and from the metal to each Fe^{++} ion. Electroneutrality is thus preserved on either side of the interface; the only region where chemical change occurs is where electricity is thus added to or abstracted from the substances in contact with the electrodes. This last is the essential condition of all electrolyses; obviously the precise character of the chemical transformations in any special instance depends on the nature of the substances present at the electrodes. Extensive molecular rearrangements may take place under some conditions; thus the anodic oxidation may result in syntheses as well as in decompositions; similarly with the reductions at the cathode. In the interior of the wire no permanent change occurs, since at each region electrons are simultaneously entering from the one side and leaving from the other in equal quantity, leaving the local condition unaltered. In the interior of the solution diffusion-changes may occur; but apparently there are no interionic or intermolecular transfers of electrons, since the quantity of electricity passing through the circuit is strictly proportional to the number of ions reaching the electrodes and charging or discharging there (Faraday's law).[1] The source of the current is thus the chemical changes taking place at the electrodes; in these changes electricity is added to the wire at one region and an equal quantity is simultaneously abstracted at another. Precisely the same chemical change takes place and the same quantity of energy is freed when the chlorine is brought directly into contact with the solution of ferrous salt; here the transfer of negative electricity from the ferrous ions to the chlorine atoms takes place without the intermediary of a metallic conductor. These considerations show that the metallic conductor, by acting as a source of electrons, merely *enables* the change of valence to take place without the direct contact of the chlorine and the ferrous salt. This effect follows because the affinity of the chlorine atom for a negative charge (*i. e.*, electron) is greater than that of the ferrous ion; the chlorine atom is thus able to abstract a negative charge from the metal and impel the ferrous ion to yield up a similar charge to it,—*i. e.*, to assume an additional positive charge and become a ferric ion. The latter then interacts chemically with whatever other substances are available.[2]

In the oxidation and reduction circuit considered above, the current on the one side of the interface, *i. e.*, in the metal, is conducted by a simple movement of electrons without chemical change; on the other side, *i. e.*, in the solution, it is conducted by the diffusion of ions and the charging or discharging of ions at the electrode-areas, with consequent local chemical change. Let us now suppose that instead of the combination of a metallic conductor and an electrolyte-solution, we have *two* electrolyte solutions separated by a thin semi-permeable partition the substance of which can combine chemically with the ions of one of the solutions. Such an arrangement corresponds to the case of the living cell immersed in its normal medium. The two solutions are to be conceived as differing in their electrolyte-content, so that a potential-difference exists across the membrane. If now we consider a circuit as arising between different regions of the surface in consequence of some local chemical change in the substance of the partition, such as an oxidation, it is clear that the conditions of the flow of current in both solutions must be in principle similar to those just considered; *i. e.*, the mode of current-transport accompanied by chemical change which prevails on one side of the interface in the example cited, *i. e.*, in medium *E*, may equally well prevail on *both* sides. The essential conditions are that the chemical changes taking place at the interface must be of such a kind as to furnish free energy (for the flow of current), and that the necessary electro-neutrality of either solution is preserved intact. It is not difficult to imagine the general features of such an arrangement, although as regards the special nature of the conditions existing in living cells (the substances involved, the nature of the reactions, etc.) little definite can be said at present. In brief, then, the present conception regards the current in the interior of either solution as transported by the movement of ions in the usual manner; but in its transport across the boundary-partition chemical reactions are concerned; *i. e.*, in this part of the circuit the essential change consists in transfers of electrons between interacting molecules or ions with corresponding chemical reactions which furnish the energy of the current. Such processes would be conditioned in the same essential manner as the processes at metallic electrodes, *i. e.*, would be of the nature of electrolysis, since they would depend upon the flow of the current through the circuit. Hence they would be influenced by electric currents from outside sources, besides giving rise to currents when they occur of their own accord under normal conditions. It seems highly probable that the characteristic electrical properties of living cells depend upon arrangements of essentially this kind; the fundamental general fact that cell-activities both give rise to electrical currents, and are themselves profoundly influenced by currents of outside origin, is most readily understood on this hypothesis.

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[1] Were this otherwise, electricity could flow through the solution by shifting of electrons from ion to ion (or molecule to molecule, etc.) without the transport of the ion as a whole. This, however, does not seem to occur (unless the current-intensity is very high). It is only when the ion interacts chemically with the substances at the electrode, or with the electrode itself, that any flow of current can take place. This is why electrolysis is always associated with the passage of a current through a solution. The metallic state appears to be characterized by a relatively high concentration and free mobility of electrons.

[2] The presence of potassium thiocyanate in the solution at the anode is a convenient way of demonstrating the production of ferric ions. The ferrous salt should be as free as possible from ferric admixture.

The special nature of the conditions in living cells may now be briefly considered, although for the present little more can be done than to indicate the probable class to which the phenomena belong; the elucidation of details is a task for future research. In general the film separating the protoplasm from the external medium is thin and semipermeable, and the solutions on either side of this partition are of different composition. Hence the conditions for a potential-difference between protoplasm and medium exist; and such a state of electrical surface-polarization is always found. This polarization varies characteristically with the nature and the physiological activity of the cell; and circuits are thus continually arising in active tissues (like muscle, nerve, etc.) between different regions of the cell-surface. The conditions under which the normal bioelectric circuits originate seem most closely analogous to those prevailing in such an arrangement as the "oxygen-hydrogen cell" of electrochemistry, in which two platinum electrodes, charged respectively with oxygen and hydrogen, are immersed in an electrolyte-solution. In this case the oxygen electrode forms the cathode, the hydrogen, the anode; the oxygen gives up positive charges to the platinum when the circuit is closed, while the hydrogen receives such charges from the metal; the oxygen thus forms oxygen- or hydroxyl-ions, the hydrogen forms hydrogen-ions; and the union of these to form water furnishes the energy of the current. Let us suppose—to make the case concrete—that the external solution bathing the living cell contains oxygen, and that the surface-film consists of oxidizable material. In the resting condition of the cell there is a certain tendency for the oxidation to take place (or oxidation-tension), *i. e.*, for the oxygen to impart positive charges to the oxidizable partition, becoming itself ionized; but this tendency is held in check—just as in the oxygen-electrode when the circuit is open—because such a transfer would leave unbalanced negative charges behind in the medium. Hence an equilibrium exists, similar to that at the surface of any battery-plate with open circuit. But any change of condition by which negative charges may be set free in the protoplasm at any region will disturb this equilibrium and allow the combination to take place. The oxygen then imparts its positive charges to the oxidizable material of the partition; this on becoming positive may then combine with the negative ions present—which may be oxygen or hydroxyl or other available anions, according to concentrations, affinities, etc.—and the material assumes a higher state of oxidation; other transformations, including syntheses, may follow from this change. Such a process implies that a positive electrical stream enters the cell-surface from the medium at the site of oxidation, and leaves the cell at other regions; at the latter regions presumably cations are given off from the cell-surface (which appears to act as a reversible electrode relatively to cations). A circuit thus flows through cell and medium, the positive stream flowing in the external medium toward the region of oxidation and away from the resting or chemically inactive regions. In other words, the local combination of oxygen with the cell-surface at any region involves the entrance of positive charges at that region, in a manner analogous to the entrance of positive charges at the cathode in the oxygen-hydrogen cell. So far as the physiological evidence extends, the region of highest oxidation-rate in cells does appear always to be negative (in the physiological sense) toward regions of lower oxidation-rate. This is indicated by the general fact that stimulated or active regions are negative relatively to inactive regions, oxygen being typically required for such activity; actively growing regions appear also to be negative relatively to less active or non-growing regions; this is seen in the regeneration of hydroid stems and of the tails of fishes, and in the growth of seedlings,[1] and is probably general, although at present the observations are insufficient to establish this generalization empirically. In such cases the negative regions—*i. e.*, the regions where the positive stream enters the cells from outside—are the regions of most active constructive metabolism; and since oxygen is required for growth and regeneration, this fact may be regarded as an expression of the general connection between oxidation-processes and syntheses. It should be added that changes in the local physical or chemical condition of the surface-film, induced (*e. g.*) by external agents, may also produce bioelectric circuits accompanied by oxidation processes; thus a region of increased permeability resulting from stimulation may be the condition disturbing the equilibrium; or a local metabolic change may take place in the surface film resulting in abstraction of cations from the cell-interior by a process of reduction; in a circuit like that imagined above such a change would result in oxidation at other regions of the cell-surface. The conditions under which local oxidations occur and bioelectric circuits originate undoubtedly vary widely in different cells, and probably also in the same cell at different times.

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[1] *Cf.* Mathews, *loc. cit.* For the case of seedlings *cf.* Müller-Hettlingen, *Pflüger's Archiv*, 1883, Vol. 31, p. 193. Hermann first observed that the growing root-tip in seedlings was strongly negative to other regions (*Pflüger's Archiv*, 1882, Vol. 27, p. 288), and Müller-Hettlingen followed the subject further at Hermann's suggestion.

It thus seems clear that electrical circuits may originate through chemical reactions which take place at the boundary between solutions of different composition separated from each other by semi-permeable partitions having the properties of the plasma-membranes of living cells. There is no theoretical necessity for a conductor of the first class. Local transfers of electricity to and from the protoplasm may occur simultaneously at two different regions of the cell-surface as the result of chemical interaction between the substance of the surface-film and ions or ion-yielding substances present in the protoplasm or in the medium; circuits between these regions thus arise. The possibility of this general condition must be admitted if we accept the general electrochemical theory that the metallic conductor in (*e. g.*) an oxidation-reduction cell enables the reaction to take place simply by serving as medium for the transmission of electrical charges. The phenomenon of chemical action at a distance appears indeed to be a clear demonstration that chemical action depends upon the transfer of such charges. The production of the bioelectric circuits becomes intelligible if we assume that the charges conditioning the reactions at the electromotor surfaces may be derived from oxidizing or reducing substances present in the protoplasm, or surroundings, just as readily as from a metallic conductor.

This general conception is evidently related to the conception of ionic exchange in such a phenomenon as adsorption.[1] Local circuits should be equally possible in such cases, and these may be associated under certain conditions with chemical change. The action of strong electrolytes upon poorly conducting material (*e. g.*, wood, rubber, leather, etc.) is probably accompanied by electrolytic action of this or similar kind. The distinction between ordinary chemical action and chemical action due to electrolysis is in fact rendered arbitrary by the facts of chemical action at a distance. Wherever electrical transfer is possible under conditions not resulting in the development of a counter E.M.F. or other polarizing effect which arrests the current, the chemical influence may be transmitted to a distance. This explains why circuits are necessary to this process. Of course the rate of any reaction thus conditioned varies inversely with the electrical resistance of the circuit which determines it; and it is only when the resistance is relatively low—as in circuits consisting of a metallic conductor combined with an electrolyte-solution, or in circuits of two electrolyte-solutions separated by a polarizable semi-permeable partition—that such transmission can take place through considerable distances.

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[1] See Höber's "Physikalische Chemie der Zelle," pp. 238 seq.

A difficulty may be felt in accounting for the conduction of electricity across the membrane in living cells. This is normally *semi-permeable*, *i. e.*, impermeable to ions and other diffusing substances; the continued existence of the cell depends in fact upon this condition. The assumption of a selective permeability to cations (or reversibility to cations) may remove the difficulty of explaining how the current can be conducted across the resting regions of the cell-surface. At the active regions conduction may take place by chemical reaction accompanied by electron-transfer, as already suggested. It seems possible, however, that at times the positive stream may pass from the cell-interior to the external medium without such chemical change; thus the addition of cations to the membrane from within, following stimulation at some other region, might disturb the polarization-equilibrium and detach corresponding cations from the outer surface; at such regions the current would flow from within outward without change in the membrane, as observed at the resting regions of the cell. In such a case the membrane would behave like a non-polarizable electrode. To what degree and under what circumstances this possibility is realized is difficult to say. Certainly in most of the instances hitherto investigated (muscle, nerve) the passage of the constant current through the cell produces marked polarization-effects (*i. e.*, sets up immediately a strong counter E.M.F.). Nevertheless the above possibility should be considered.

V. General Conclusions.

The foregoing discussion raises the question whether in organic growth the essential structural condition is not the presence of semi-permeable and hence electrically polarized partitions separating the living substance from its medium, and at which processes of electrolysis may take place. If this is so, the prevalence of the cellular type of organization would be largely accounted for. Organic growth (as well as normal maintenance) would then involve the deposition in the continuous surface-layer of materials which preserve the character of that layer, *i. e.*, its continuity, semi-permeability, and specific physico-chemical composition. It is clear that any layer having such properties would have to form a *closed surface;* otherwise the difference between the inner and outer solutions could not persist; hence a "cell"—mass of protoplasm enclosed by a membrane—would be the living unit. A tendency to a continual increase of this surface-layer in an appropriate nutrient medium would then constitute the primary feature of the growth-process. Evidently the continued growth of such a system would involve a decrease in the ratio of surface to volume: and the need of preserving this ratio above a certain minimum may be the condition that has led to the association of cell-division with growth, as Herbert Spencer suggested long ago. According to this conception, "living" material would be essentially material at the boundary-surface or under the direct influence of the boundary-surface; definite limits would thus be imposed upon the dimensions of the individual living units. In general we find that protoplasm is partitioned freely; *i. e.*, the ratio of surface-protoplasm (membrane-protoplasm) to the total mass is usually large. The exceptions (yolk-laden eggs, muscle cells, certain plant-cells) may be explained on special grounds.

There is no doubt that the distinctive vital properties of self-maintenance, growth, and reproduction are closely interconnected; it would probably be more correct to say that they are manifestations of the same fundamental physiological activity under somewhat different conditions. The essential feature of this activity is constructive metabolism of a specific kind. The mechanism determining this process of construction must be intimately connected with the mechanism determining the response to stimulation, since stimulation, which effects a breakdown of the living substance, must itself form the condition of the restitution of the broken-down material, if the system is to continue to exist. This was clearly recognized by Claude Bernard.[1] Some kind of self-regulating cycle, in which a destructive process calls forth automatically or inevitably a counter-process of repair, would thus seem to be the necessary foundation of any form of vitality. An analogy to this condition is seen in the chemical processes of any electrolytic circuit; just as oxidation at the one electrode involves the inverse process at the other electrode, so decomposition or structural breakdown at one region of the cell-surface may, by giving rise to a local bioelectric circuit, directly determine a reparative synthesis either at the same or at an adjoining region.[2] Some general physico-chemical condition of a simple kind must be common to *all* of the various types of living system, since they are all *living*. The universality of the cellular type of organization seems—to my mind at least—to indicate that the essential structural condition in living matter is the presence of polarizable partitions at which processes of electrolysis, including both syntheses and decompositions, are to be conceived as taking place under the influence of local electrical circuits. The self-maintaining character of the living system, its responsiveness to outside influence (especially of an electrical kind), and the transmission of chemical influence to a distance, become to a considerable degree intelligible on such an hypothesis. These considerations may appear too speculative to many; but further advance in physiological analysis seems to demand that the fundamental vital processes should at least be assigned to their proper physico-chemical classes. The similarities between the phenomena described in this paper and many of the most characteristic peculiarities of the growth-process in organisms seem to me to be too detailed not to signify an identity in some essential underlying condition. The need of a physico-chemical analysis of the growth-process is clear; growth is unquestionably the most fundamental of vital activities, and any adequate theory of growth would be equivalent to a theory of the vital process itself.

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[1] *Cf. e. g.*, "Leçons sur les phénomènes de la vie," Vol. 1, p. 127.

[2] For further discussion of this possibility *cf.* my recent paper, *Amer. Journ. Physiol.*, 1917, Vol. 43, pp. 56, 57.

THE CONCRESCENCE OF FOLLICLES IN THE HYPOTYPICAL OVARY.[1]

LEO LOEB.

In a preceding paper we have shown that lack of sufficient nourishment is the cause of a hypotypical condition of the ovaries. In one guinea pig we found in addition to the hypotypical condition another peculiarity of the ovaries, which is of considerable interest and which deserves a special description. In connection with the facts reported in the preceding paper it leads to some interesting conclusions as to the effect of underfeeding on the development of the ovarian stroma, and on the development of what in the guinea pig in certain respects corresponds to the interstitial gland of the rabbit and it may throw light on the origin of follicles containing more than one egg.

Female guinea pig No. 383 was obtained from Iowa. On January 12, 1917, it weighed 475 grams; on that date both lobes of the thyroid of this animal were removed. One thyroid lobe from another guinea pig, obtained from a different breeder and weighing 380 grams, was transplanted into the subcutaneous tissue of guinea pig No. 383. The transplanted thyroid was removed for microscopic study seventeen days later, January 29. At that time the guinea pig weighed 322 grams; it had therefore lost 32 per cent. of its original weight in a period of seventeen days. The transplanted thyroid was on the whole in a very good condition; we shall refer to this aspect of our findings in another connection. Here we are especially concerned with the condition of the sexual organs. The uterus shows low or medium cylindrical epithelium of the surface and glands with a very small number of mitoses in the surface epithelium. The mucosa is thin and fibrillar but very hyperemic. In the lumen of the uterus there are some erythrocytes. The ovaries are markedly hypotypical. They contain several atretic yellow bodies, the remnants of former corpora lutea. There are in the cortex a number of primordial follicles developing into small Graafian follicles and into follicles of small to medium size. But before they have hardly reached the latter size, a slow solution of the granulosa sets in and very soon the cavity of the follicles is lined by the theca interna.

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The further course of the atresia of the follicles is, however, changed; the connective tissue does not as usual grow into the follicular cavity; instead the atretic follicles present themselves as small cysts. The ordinary follicles in various stages of connective tissue atresia are therefore lacking. There is, however, present a very large number of follicles in the last stage of atresia, a cavity with remnants of the zona pellucida, or merely a small cavity surrounded by a thick layer of the shrunken theca interna cells. By far the greater part of the ovaries consists of such follicles in the last stage of atresia; the proportion of such follicles is much greater in this animal than in normal ovaries which are in the period following the first week of ovulation and even larger than in the other hypotypical ovaries which we observed.

There are two further peculiarities noticeable in these ovaries, viz., (1) a relative decrease in the amount of fibrous tissue which surrounds the various follicles, and (2) the presence of numerous follicles with multiple eggs. While we observe around a number of growing or atretic follicles a fibrous membrane, the latter is always thin andit is absent around other follicles. Not rarely we see two adjoining follicles of small to medium size merely separated by several rows of relatively large theca interna cells; or we see small follicles close together, and not separated by fibrous tissue; at other places the theca internae of two quite atretic follicles press on each other, the theca cells of the one follicle surrounding directly the capillaries of the theca interna of the adjoining follicle. In other cases two or three shrunken egg cavities are enclosed in one mass of small theca interna cells.

If we compare the amount of fibrous tissue separating the follicles in this animal and in other animals with either normal or hypotypical ovaries, we find in the ovaries of other guinea pigs a larger amount of fibrous stroma separating the various follicles. This decrease in fibrous tissue made it possible to differentiate microscopically the ovaries of this animal from those of other guinea pigs. There occurs in these ovaries a considerable number of follicles with two, three or even more eggs. We see in the most peripheral zone of the ovary two well preserved eggs of about the same size, but one slightly pressing upon the other and both surrounded by a single layer of granulosa cells. Somewhat further away from the tunica albuginea we see small follicles with two eggs of the same size, separated only imperfectly by a few flat granulosa cells. At other places we see follicles of small to medium size with a cavity. At the end of the cavity directed toward the center of the ovary there is a relatively large, well-preserved egg, surrounded by several rows of granulosa cells. At the side of the follicle directed toward the periphery of the ovary there is a very small, but well-preserved egg, in an earlier stage of development, surrounded by only one layer of granulosa cells. Here we have a combination of a primordial and of a small Graafian follicle. In other cases we see a small egg of a primordial follicle, pushing its way through the granulosa of the somewhat larger follicle and just reaching the cavity with the outer pole; the most frequent orientation of the two eggs is as follows: The larger one, surrounded by several rows of granulosa cells, is placed at the central pole of the cavity and the smaller one more toward the peripheral pole. But occasionally the order may be reversed, or the small egg may be at the lateral aspect of the follicle. In one case we found the egg of the primordial follicle in the suspensory ligament of the granulosa of the larger follicle. In still other cases we find a combination of a small egg at an early stage of its development, surrounded by one row of granulosa cells, the whole corresponding to a primordial follicle in the cavity of a small to medium follicle in which the granulosa and egg have already undergone degenerative changes. The degenerating ovum may still be surrounded by granulosa cells or the granulosa cells may have been dissolved. In other cases we find in a follicle of small to medium size two well developed eggs, both surrounded by several layers of granulosa cells. Similar combinations we find in follicles with three eggs. There may be combined a degenerated egg, a relatively large, preserved egg surrounded by several layers of granulosa, and an egg of a primordial follicle enveloped by one layer of granulosa. In a follicle in which we found a still larger number of eggs, the latter were well preserved and rather small, and situated in different parts of the granulosa. Of interest is also an observation which showed that a disintegrating egg may enter into direct contact with the theca interna of a nearby follicle of small to medium size.

These are the principal combinations of double or multiple eggs we observed in the ovaries of this animal, and both ovaries behaved in a similar manner. How shall we interpret this condition? The large number of follicles with two or more eggs which we found in both ovaries indicates clearly that we have to deal with a general condition of the organism affecting both ovaries equally and not with a local ovarian change. We may conclude that bioval or plurioval follicles may originate in either of the two following ways: (1) The young eggs in the tunica albuginea of the ovary remain united; connective tissue fails to grow between two or more eggs and to separate from each other the different eggs with the surrounding granulosa cells. The pictures of primordial follicles containing two eggs speak in favor of this interpretation. (2) But other follicles containing more than one egg must originate in a somewhat different manner. Very small follicles, especially primordial follicles, push their way into larger follicles, perhaps even into such follicles which are already in the process of degeneration. And this is probably by far the more frequent mode of origin. We find different stages in the junction of two follicles of different sizes, and in different stages of development. We can follow the pushing in of the smaller into the larger follicles. The fact that the thickness of the granulosa surrounding the different eggs in a follicle is in accordance with the character of the egg is only compatible with the view that two formerly distinct follicles effected a secondary union. In favor of this interpretation speaks also the observation that in the majority of cases the more undeveloped egg is situated toward the outer pole of the larger follicle. This is in accordance with the fact that the smaller a follicle is, the more peripheral is its situation. Occasionally, however, a primordial follicle may be pushed deeper into the cortical tissue before it enters the wall of the larger follicle. In this case we might expect to find the smaller egg in the central part of the follicle and the larger one more toward the periphery. Whether also two small to medium follicles of similar size can unite to form one follicle with two eggs, we are unable to determine with certainty.

Essentially both modes of origin are in all probability based on the same condition, both being due to the same cause, namely, the relative inactivity of the connective tissue in these ovaries. We saw that in this animal the atresia of the follicles does not take its normal course, but that after disintegration of the follicles the connective tissue fails to grow into the cavity and that thus small follicular cysts syrrounded by theca interna are formed. We saw furthermore that the fibrous bands separating the various follicles are not so well developed as in the ovaries of other guinea pigs. It is therefore very probable that this deficiency in the connective tissue is at the bottom of this condition. The relative inactivity of the connective tissue, its failure to proliferate and to produce fibrous tissue, accounts for the imperfect atresia of the follicles and the imperfect separation of the follicles, and it is this inactivity of the connective tissue which is responsible for the occurrence of the follicles containing multiple eggs. In the first place, the connective tissue in some cases fails to grow properly between the very young eggs and their granulosa and to separate the two follicles; thus the first mode in the production of follicles with several eggs is brought about. It is due to a pathological persistence of an earlier stage in the development of follicles. And secondly the lack of development of strong fibrous tissue around a number of follicles, together with the pressure existing in the ovary as the result of the growth of young follicles, is responsible for the pushing of small follicles into larger ones. Thus there is no essential difference in the mode of origin of bioval or plurioval follicles at various stages of development.

The next question concerns the cause of this relative inactivity of the connective tissue. The study of the hypotypical follicles, which we reported in the preceding paper, throws light on this problem. We mentioned there that in cases in which the hypotypical condition was pronounced, there was noticeable a tendency to delay in the ingrowth of connective tissue into the follicular cavity; thus small cysts resulted. We interpreted this relative inactivity as due to the underfeeding which at last affects also the connective tissue. This condition represents a further stage in the chain of changes produced through underfeeding. This chain is as follows: (1) The maturation of follicles is suspended. (2) Abortion takes place in pregnant guinea pigs. (3) The granulosa cells of developing follicles are injured and are prematurely dissolved, but the relative strength of new formation on the one hand, and of destruction of granulosa cells on the other hand, shows such a balance in favor of new production that the development of follicles progresses so far that medium follicles are formed. (4) This balance becomes more unfavorable and only small and small to medium follicles develop; after this stage has been reached and the granulosa has been dissolved connective tissue grows in and fills the cavity and leads to a shrinking of the follicle. (5) In the last stage even the connective tissue becomes inactive. The ingrowth of connective tissue into the cavity does not take place. And the same process leads also in all probablity to an under-development of the fibrous bands separating the various follicles. Thus the separation of follicles becomes interfered with and the multiplicity of eggs in a follicle results.

It is, of course, possible that there are at work additional factors which favor such a process. It might be that in certain individuals the tendency of connective tissue to be inactive is greater than in others. It might also be that the number of the young follicles in early stages of development is greater in this animal and that thus a crowding of young follicles results. However, if such a difference exists between this and other ovaries, it is not very marked. Multiplicity of eggs in the ovarian follicles has been observed by us in several other ovaries of guinea pigs. At the time however when our previous observations were made we had not yet gained an undestanding of the connection between this change and the abnormal conditions which we found in this case. Ovarian follicles with several eggs occur also in other species, especially also in man. It would be of interest to inquire whether in all cases the causes are the same. or whether different conditions may lead to the same result; In such an inquiry we have to keep in mind the possibility that a hypotypical condition of the ovaries might be a transitory state; that after having led to the production of follicles with multiple eggs, new follicles may again begin to develop normally, so that in the end nothing indicates that at one time such ovaries had been hypotypical.

This concrescence of follicles is somewhat analogous to a condition we find in other glands. In the thyroid of the guinea pig, as well as in the mammary gland of the mouse, we found occasionally structures which could only be explained as due to a concrescence of neighboring acini; due to the disappearance of the walls separating adjoining acini. We have therefore to deal with a phenomenon of a more general character, which under certain conditions may perhaps occur in all the glands.

There was in the ovaries of this guinea pig an additional feature which deserves special mention, viz., the very marked development of what corresponds to the interstitial gland in the rabbit. As we stated above, by far the greater part of this ovary consists of follicles in the last stage of atresia, follicles in which the shrunken cells of a well-developed theca interna surround one or several small cavities, the remnants of what were formerly follicular cavities. While we find in all hypotypical ovaries a relative preponderance of this kind of follicle, in the ovaries of this guinea pig this feature was more prominent than in the ovaries of the other animals. This is due to the following factors: (1) At the time of the thyroidectomy, the ovaries were in all probability relatively large, the animal weighing at that time 475 grams; thus space was available for the expansion of atretic follicles. (2) The follicles ceased to develop to medium or large size. Thus the pressure exerted by the larger follicles was eliminated, and a chance was given the atretic follicles to occupy the space otherwise occupied by large follicles. The slight development of fibrous bands around the follicles must have a similar effect diminishing pressure exerted on atretic follicles. We may therefore conclude that the structure analogous to the interstitial gland is prominent whenever the intraovarian pressure is relatively slight. Under these circumstances the last stage of follicular atresia is preserved throughout a relatively long period of time. A similar condition we find in the ovaries of the guinea pig within the first week after ovulation, when the larger follicles have all become atretic.

Conversely we may conclude that the intraovarian pressure hastens the disappearance of the theca interna and of the other vestiges of atretic follicles. The pressure of the growing structures is stronger than the pressure of the resting and disappearing structures, and thus the life and growth in one part of the ovary hastens the disappearance of degenerating structures elsewhere. Not only is the disappearance of the shrunken theca internæ thus hastened, but also that of the fibrous tissue structures in the ovary. In our case it is uncertain whether the connective tissue had formerly grown into the follicles which are now in the last stage of atresia, this connective tissue having been absorbed subsequently, or whether in this animal the last stage of connective tissue atresia was reached directly from the cystic condition through absorption of the fluid in the follicular cavity, without a preceding ingrowth of fibrous tissue. In case the latter alternative should hold good the lack of ingrowth of connective tissue into atretic follicles would have been present for some time previous to the examination of the ovaries. In some atretic follicles we notice however some vestiges of fibrous tissue around the central cavity and it is therefore probable that in these cases the connective tissue had previously grown into the cavity after the solution of the granulosa. We also must consider the possibility that diminished intraovarian pressure may in itself influence the activity of the connective tissue.

Summary.

We describe the ovaries of a guinea pig in which the experimentally produced hypotypical condition was more advanced than in any of the other animals which we observed. In this case the underfeeding not only affected the activity of the granulosa, but also of the connective tissue. In consequence of a lowered activity of the connective tissue a concrescence of follicles takes place in numerous cases in both ovaries. A lowering of intraovarian pressure in such ovaries leads to a relative preponderance of what corresponds to the interstitial gland in the ovaries of certain other species. The concrescence of ovarian follicles is analogous to the union of neighboring acini in the mammary gland and thyroid. It is a phenomenon which occurs probably in all or the majority of glandular structures.

A COMPARISON OF MITOCHONDRIA IN PLANT AND ANIMAL CELLS.[1]

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The recent discovery, through the elaboration of new methods of technique, of the wide distribution of mitochondria has attracted the attention of many investigators in widely separate fields. Their characteristic form, strongly suggestive of bacteria, is now quite familiar. The technique was devised by anatomists and was applied by them to plant cells. I refer particularly to Meves' ('04, p. 284) work on *Nymphæa*. But, as might have been expected, these structures did not altogether escape the careful scrutiny of the older botanists, despite their imperfect methods of technique; for Zimmermann ('93, p. 215) certainly observed and described mitochondria in the living hair cells of *Momordica elaterium* and in the meristem and root tip of *Vicia faba*. Nevertheless botanists in this country have been slow to study mitochondria notwithstanding the fact that in properly made preparations they constitute a cell organ as conspicuous as the nucleus.

Unhappily we have deplorably little experimental evidence to lead us to any conclusion as to their functional significance in the cell economy. Our curiosity has been so insistent that we have resorted to less reliable sources of information, one of which is the argument from analogy. It is said that since the mitochondria are found in almost all active cells, their function must be a generalized one which they all possess in common. The validity of this line of reasoning rests entirely upon the resemblance of mitochondria in these different cells. Mitochondria, however, differ slightly in their solubility in acetic acid and in other respects, and in direct proportion to the extent of variation the above argument loses force.

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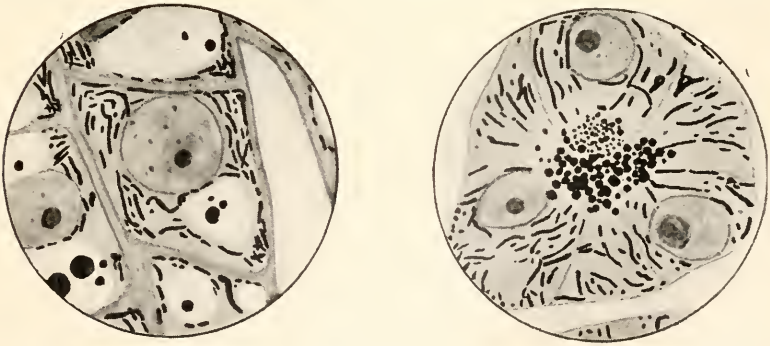
[1] This work was also carried on at the Marine Biological Laboratory, Woods Hole, Mass., where the Director, Dr. Lillie, very kindly placed a research room at my disposal. It was aided by the Carnegie Institution of Washington.

A very important issue at stake is the relationship of plant and animal mitochondria. Are the researches of the botanist of interest to the anatomist and vice versa? Should we attempt to coördinate and correlate the two, and, if so, how far can we go? Though these questions have never been directly attacked, considerable difference of opinion is apparent in the literature. Suffice it to say that Pensa ('14, p. 22) states that the mitochondria in plant and animal cells are not identical; Guilliermond ('13*a*, p. 481) and Smirnow ('06, p. 153) think that they are; Duesberg and Hoven ('10, p. 99) believe them to be homologous; while Sapěhin ('15, p. 320) holds an intermediate view. He thinks that in the higher plants mitochondria-like forms may be divided into two groups: "plastids" and "chondriosomes" which, however, are indistinguishable in the early meristem.

I have devoted my whole attention to this single problem of the relationship of plant and animal mitochondria with the hope of obtaining results which are clear-cut, concise and definite, bringing to bear upon it old, as well as entirely new, methods of technique in the form of supravital dyes of the janus green series which have never before been applied to plant cells.

Observations.

The statements concerning the similarity or the dissimilarity of plant and animal mitochondria have been made, with but few notable exceptions, by investigators having personal experi-



Figs. 1 and 2. Cells from the pea and the pancreas fixed in formalin and bichromate, mordanted in bichromate (Regaud IV. *B*) and stained with iron hematoxylin. (1,500 diameters.)

ence with one or the other, seldom with both. It is essential that the two should be compared side by side.

I have chosen for my experiments the cells of the radicle of the pea and the acinus cells of the pancreas of the mouse on account of the close resemblance of their mitochondria. The general appearance may be seen by reference to Figs. 1 and 2. Morphologically the mitochondria would seem to be identical in the two; but in reality this is not the case, for I have examined them very carefully with a high magnification (3,500 diameters) and I find that they are, on the whole, slightly longer and thicker in the pancreas. I have made detailed drawings to scale of selected mitochondria from both. This can be done with considerable accuracy by making use of a good camera lucida, with appropriate illumination, and a sharply pointed pencil. The results are illustrated in Figs. 3 and 4. Filaments, rods and granules predominate. Branching filaments, networks, spherules and so on are rarer, but occur in both. In fact no form, however bizarre, is to be found in the one for which a counterpart cannot

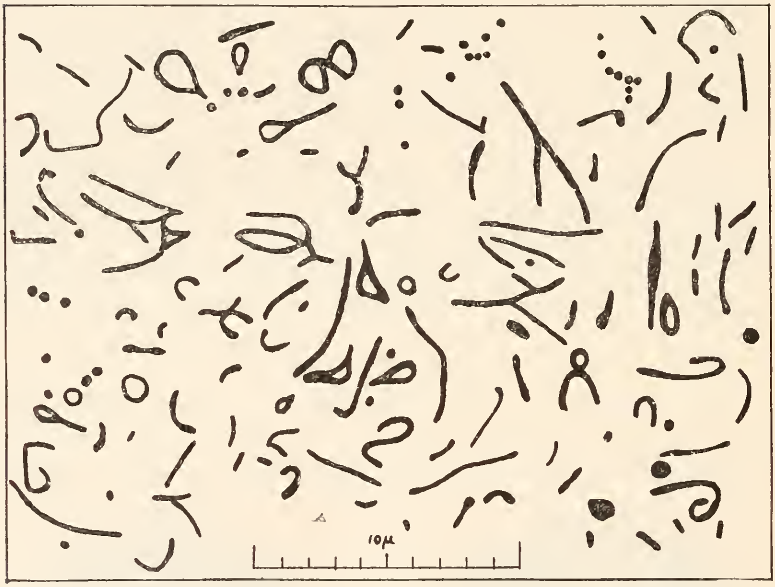


FIG. 3 Selected mitochondria from cells of pea radicle prepared by Regaud IVB to show the extent and limits of the polymorphism. (3,500 diameters.)

be discovered in the other. The absence of very minute mitochondria, merging into the invisible, is of interest from the point of view of an origin *de novo* through condensation; and the polymorphism, in both the pea and the pancreas, makes plain the hopelessness of any attempt to devise a system of individual nomenclature, based upon morphology, to embrace all the forms. Obviously the material selected permits of very close comparison.

I have taken precautions in the following experiments to eliminate, as far as possible, chance variations due to incurrent experimental errors which would, however, in any event, be likely to effect both tissues similarly (see p. 207). They were fixed together in the same bottle, dehydrated together, cleared together and embedded in the same block of paraffin together. They were cut with the same sweep of the knife, mounted on the same slide and stained at the same time. Many preparations have been made and in each of them the dehydration, clearing and embedding were carried on in the same way, so that the results are comparable. The sections were stained with iron

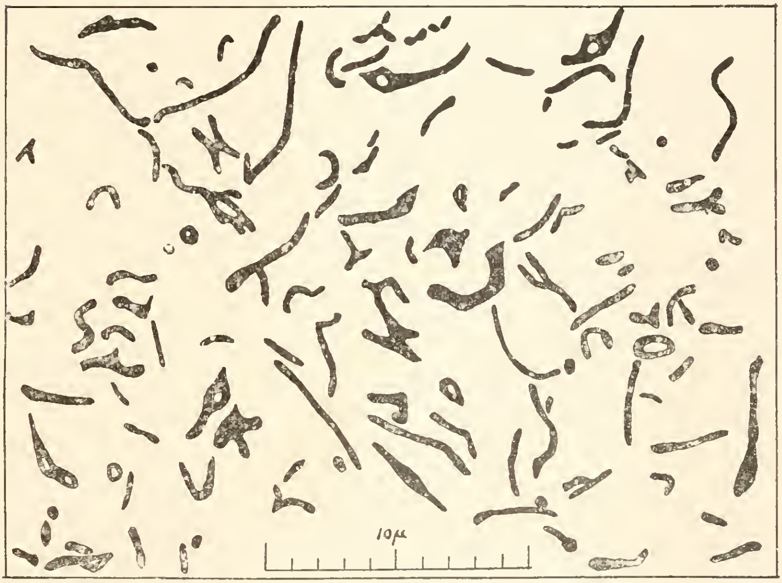


FIG. 4. Selected mitochondria from acinus cells of the mouse, treated in exactly the same way, showing similar polymorphism. (3,500 diameters.)

hematoxylin, with fuchsin methyl green, with Altmann's fuchsin picric acid and sometimes by the Benda method. The chief results are briefly set forth in the following table, but a large number of additional controls were made.

In a general way, running our eyes down the table, we see that the response of mitochondria to fixatives is the same in plants as in animals. Similar fixations preserve them, modify them and destroy them in much the same way in both. It also shows that the technique is easy, not difficult, and that a number of chemicals, which are generally thought to be destructive, will fix them more or less satisfactorily.

The standard mitochondrial fixatives, like Regaud ('10, p. 296) II., III., IV. *A* and IV. *B*, Benda's modification of Flemming's fluid, the acetic acid osmic bichromate mixture of Bensley, and Zenker, without acetic, will fix them in both tissues. I have found, however, that Regaud's mixtures are by far the most satisfactory because they do not produce the artificial coagulation of the ground substance caused by the others. They also preserve the true form of the mitochondria more faithfully; though I have reason to suspect that in some cases, in plant cells, they cause fragmentation and shrinkage. I have studied the behavior of the ingredients of these fluids.

Formalin is perhaps the most important since it penetrates rapidly, retains the homogeneous appearance of the ground substance and preserves the mitochondria satisfactorily. Formalin alone (Sapěhin, '15, p. 321) serves as an excellent fixative for mitochondria. My best results were obtained with a solution of formalin of from 5 to 10 per cent. made up from ordinary commercial formalin neutralized with magnesium carbonate. More dilute solutions tend to cause a swelling of the mitochondria and more concentrated ones a shrinkage; but different tissues require different concentrations. Regaud advises a subsequent mordanting with bichromate, but I find that this is quite unnecessary. Used in combination with 3 per cent. potassium bichromate, according to the directions of Regaud, formalin gives the best of fixations with both the pea and the pancreas, though in my experience the results may be even improved by diluting the mixture with an equal volume of water as advised

TABLE I.[1]

No.	Fixative.	Radicle of Pea.	Pancreatic Acini.
1615B..	5% formalin 41 days	+normal	+normal
1574A..	5% " 4 "	+normal	+normal
1575A..	10% " 4 "	+normal	+normal
1616A..	40% " 4 "	+irregular, shrunken	+nodular, shrunken
1580....	2.5% " 4 " mordanted in 3% bichromate for 21 days	+but slightly swollen	+swollen and fragmented
1574....	5% "	+normal	+normal
1575....	10% "	+fragmented	+few
1576....	20% "	+normal	+few
1616B..	40% "	+normal	+normal
1585....	formalin 10 c.c., saturated aq. picric acid 40 c.c. (Regaud II) 1 day	+fragmented	+normal, indistinct.
1585B..	same, mordanted in 3% bichromate 20 days (Regaud III)	+normal, unusually filamentous	+normal, indistinct
1577A..	5% formalin, 10 c.c., sat. aq. picric acid 40 c.c., 1 day	+fragmented, distorted	+fragmented
1577....	same, mordanted in 3% bichromate for 16 days	+fragmented, distorted	–
1578....	10% formalin, 15 c.c., 1% chromic, 85 c.c.; mordanted in pyroligneous 1 day and 2% bichromate 1 day	+fragmented	+few and fragmented
1566....	formalin 20 c.c., 3% bichromate, 80 c.c.; mordanted in 3% bichromate 15 days. (Regaud IV*B*)	+normal	+normal
1628 ...	Formalin 10 c.c., sat. aq. corrosive sublimate 40 c.c. 1 day	–	–
1629....	Formalin 10 c.c., sat. aq. corrosive sublimate 20 c.c., sat. aq. picric acid 20 c.c. 1 day	–	–
1582....	Boiling water 5 min.	–	–
1583....	95% alcohol	–	–
1447....	Gradual increase in concentration of alcohol, 2.5, 5, 7.5, 10, 15, 20, 30, 40, 50, 70, 80, 95% and absolute; chloroform	–	–
1584....	95% alcohol 10 c.c., 3% bichromate 10 c.c. 4 days	–	–
1618....	2% osmic 2 c.c. 2.5% bichromate 8 c.c., acetic 1 drop	+indistinct	+normal
1609....	sat. alcohol sol. corrosive sublimate	?	–

"+" signifies present, "–" absent and "?" doubtful.

TABLE I—*Continued.*

No.	Fixative.	Radicle of Pea.	Pancreatic Acini.
1610....	sat. aq. corrosive sublimate	+distinct, shrunken	+few, indistinct
1611A..	sat. aq. picric acid	+nearly normal	+few, fragmented
1612....	2% chromic acid	+somewhat distorted	+few, fragmented
1613....	3% bichromate	+normal	+nodular rods, rings, granules
1614....	.5% osmic acid	+unsatisfactory	+normal

by Sapěhin ('15, p. 321). Formalin in combination with picric acid is a somewhat erratic fixative, very excellent in some instances and bad in others. In good preparations the mitochondria appear very large and not so shrunken as when acted on by other fixatives. Formalin and chromic acid constitute a poor fixative which fragments the mitochondria and, in some cases, destroys them entirely.

Potassium bichromate used alone (Sapěhin, '15, p. 321) is a good preservative for mitochondria but penetrates badly. Regaud recommends its use in mixtures and as a mordant in order to render the mitochondria insoluble in the alcohols during dehydration. The bichromate which remains in the tissue when it is sectioned, increases its affinity for fuchsin when the fuchsin methyl green stain is applied.

Ethyl alcohol in 95 per cent. solution destroys the mitochondria in both the pea radicle and the pancreas. When alcohol is employed in gradually increasing concentration the same result is obtained. When it is combined with potassium bichromate the mitochondria are also destroyed. In fact our conclusion can be none other than that alcohol, in whatever combination, is a very poor fixative for mitochondria.

Boiling water preserves the general form relations but does not bring to light any mitochondria.

Picric acid is a fairly good fixative for mitochondria in the cells of the pea and pancreas, when used in a saturated aqueous solution. It has the great advantage that it penetrates well.

Chromic acid alone causes considerable distortion of the mitochondria and somewhat irregular coagulation of the ground substance but in combination it is apparently very efficacious as shown by its inclusion in Benda's modification of Flemming's fluid and in other mixtures.

Osmic acid is the best preservative of mitochondria and the worst penetrator and is therefore useless in the cells of the pea radicle on account of their impermeable cellulose walls; yet it is this quality of being an excellent preservative which makes it so valuable in association with other chemicals. It does not cause any artificial coagulation of the ground substance.

Corrosive sublimate in saturated aqueous solution also penetrates the pea radicle very poorly; the pancrease not so badly. However it preserves the mitochondria in the pea far better than in the pancreas in spite of the fact that they are, in both cases, somewhat shrunken. In alcoholic solution it is destructive, but it is difficult to tell whether this is due to the alcohol or to the increased amount of sublimate. In Zenker's fluid the injurious action of the sublimate is counter-balanced by the presence of potassium bichromate.

The action of acetic acid is so important that I have made a detailed study of it as set forth in Table II.

In the first place I have varied the concentration of acetic acid in Regaud's mixture IV. *B* from 0 per cent. to 10 per cent. With no acetic acid the mitochondria are normal; while with a concentration of 2.5 per cent., or more, they are destroyed in both tissues, showing that their degree of solubility is the same.

I have also experimented with Zenker's fluid and I find that, with it, the mitochondria are on the whole much more resistant to acetic acid. This may be due to the fact that the Zenker's fluid was only applied for one day, instead of for five days like the Regaud's fluid; or the presence of sublimate in the Zenker's fluid may have tended to counteract the action of the acetic acid. The mitochondria are fairly well preserved when no acetic acid has been added to the Zenker's fluid. With increase in acetic acid, up to 10 per cent., they become destroyed in the deeper parts of the tissue, especially in the pea; while they are fairly well preserved in the most superficial cells in both. In the pea, also, the mitochondria are far more prone to become fragmented. This difference between surface and interior seems to be a question of penetration rather than of a difference in the mitochondria themselves. There is a simultaneous action of all the ingredients of the fixative on the cells at the surface and here the

TABLE II.

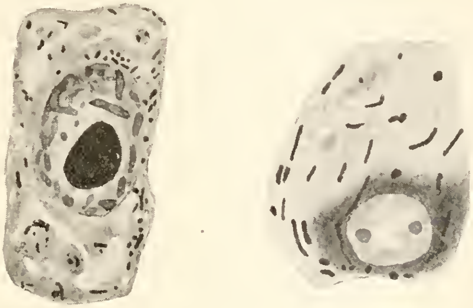
No.	Fixative.	Radicle of Pea.	Pancreatic Acini.
1570....	Zenker 0% acetic 1 day	+better preserved in older cells, apt to be vesicular, fragmented	+normal
1571....	" 2.5% "	+better preserved in older cells, apt to be vesicular, fragmented	+normal in peripheral cells, absent in center of tissue
1572....	" 5% "	+almost all destroyed in younger cells, still fairly numerous in older ones	+fewer, only in peripheral cells slightly fragmented
1573....	" 10% "	+more fragmented and vesicular	+same
1617....	" 20% "	+fairly well shown in all cells	+slightly fragmented, present in all cells
1627....	" 33% "	+but very rare	+but very rare
1626....	" 50% "	−	−
1566....	Regaud IV*B*, 0% acetic 5 days, mordanted in 3% bichromate 8 days	+normal	+normal
1567....	Regaud 2.5% acetic 5 day	−	−
1568....	" 5% "	−	−
1569....	" 10% "	−	−
1625....	Equal parts acetic and water	−	−
1624....	Pure acetic	−	−
1565....	Benda no acetic 8 days	+rather vesicular	+normal
1579....	Benda, 0.8% acetic, mordanted in formalin 20 c.c.; 3% bichromate 80 c.c. 6 days	+normal few	+normal
1579B..	Same but mordanted in pyroligneous 1 day and 2% bichromate 2 days	+normal few	+normal

bichromate, and possibly also the sublimate, modify the action of the acetic acid. In the deeper layers of the tissue, on the other hand, we have to do with a successive action of the said ingredients, determined by their relative rates of penetration. The acetic acid penetrates first and acts upon the mitochondria before the bichromate and the sublimate have come upon the scene.

Mitochondria are surprisingly resistant to higher concentrations. They are well preserved by a mixture of Zenker's fluid and 20 per cent. acetic acid and their relations are shown in Figs. 5 and 6. With 33 per cent. acetic acid only a few scattered mitochondria remain, while they are completely destroyed by a mixture containing 50 per cent. of acetic acid.

Pure acetic acid in half concentration and in full concentration destroys the mitochondria in both tissues. But it must not be thought that acetic acid in small concentrations is to be avoided because I have found that Benda's fluid without acetic acid does not seem to act so well as with acetic acid, in the amount prescribed.

In other words acetic acid exercises a solvent action on mitochondria which is the same in plants as in animals and varies with the distance of the cells from the surface and with the character of the other components of the fixative. There is reason to believe, further, that the resistance of mitochondria in certain young plant cells is less than in old ones, calling to mind the condition which obtains in the spermatogenesis of many animals where the mitochondria in the young cells are more susceptible to acetic acid than in the mature spermatozoa (Regaud, '08, p. 661); which comparison makes the resemblance of plant and animal mitochondria, in this respect, still closer.



FIGS. 5 and 6. Cells from the pea and the pancreas fixed in Zenker's fluid containing 20 per cent. of acetic acid and stained with fuchsin and methyl green. (1,500 diameters.)

I have attempted to ascertain the experimental error in the study of mitochondria in fixed tissues in plants and animals and to see whether it is the same in both.

The conditions of the experiment are not so important as the nature of the fixative but they must nevertheless be taken into consideration. Variations in the temperature (under say 46° C.) and the illumination of the tissue prior to fixation do not seem to influence the mitochondria for I have grown peas at room temperature and in the refrigerator (8 to 12° C.); in bright light and in darkness without bringing about any noticeable change in their mitochondria. Some fixatives are rather unstable and cannot be kept, even for a few hours, in bright sunlight or in a warm place. Under these conditions Regaud's formalin and bichromate mixture, for example, undergoes a rapid change characterized by a darkening in color, but its action does not seem to be impaired, though, to be on the safe side, one should avoid it. On the other hand, mechanical manipulation of the tissue before fixation often causes very confusing alterations in the mitochondria especially in the softer animal tissues.

The distribution of mitochondria within the cell is not altered appreciably in either plants or animals by the technique used, except in instances where mitochondria are present with different solubility, some being preserved and others being destroyed. This is of common occurrence in the pea radicle where with some fixatives, filaments only appear; the granules being obliterated so that the apparent distribution is altered.

The technique makes a very great difference in the number of mitochondria in the preparation. Some ingredients of the fixative are particularly likely to destroy them, like the acetic acid already mentioned; so that in the study of unfamiliar tissues we must assure ourselves that the technique is adapted to show all the mitochondria present.

The shape of mitochondria is so easily modified by the fixative that we must be on our guard here also, especially in plant cells. That is to say, we frequently meet with a fragmentation of mitochondrial filaments, the filaments of the living cell appearing in the form of rows of granules in the fixed preparation. The reverse change never takes place, for filaments are never formed through a coalescence of granules, under the influence of the fixative. Poor penetration of the fixative in the deeper layers of the tissue often causes the mitochondria to lose their characteristic form and to swell up into large spherules or vesicles. Similarly if a film on the surface of the tissue is allowed to dry, before fixation, the mitochondria in it will be profoundly modified.

The size of mitochondria is also subject to some modification, as shown by the comparison of mitochondria in living cells with those in fixed tissues. The general tendency of fixatives is to shrink them and to make them uniformly smaller than they really are in the living condition. This tendency is very slight, almost negligible, in the case of the best fixatives. On the other hand, acetic acid, and possibly also formalin in very low concentrations, have a marked swelling effect.

It must be admitted that variations sometimes do occur in the mitochondria in our preparations, particularly in the pea, for which no reasonable explanation can be advanced on the basis of the technique. They may result from rhythmical variations in the activity of the cells which we have reason to believe occur, just like periodicity in cell division, many cells dividing almost synchronously (Kellicott, '04, p. 531) but they cannot be wholly explained in this way. They may result also from the conditions under which the radicle is growing, upon whether it is submerged or exposed to the air; but I find that radicles grown under exactly the same conditions occasionally show mitochondrial variations which are very perplexing.

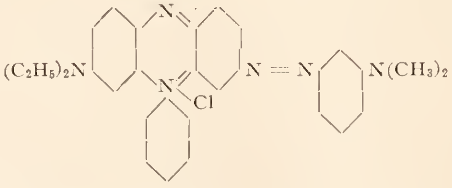
Evidently the experimental error is something which we must have considerable respect for, but which can be controlled if the proper precautions are taken. Its identity in plant and animal tissues is another indication of the similarity of the mitochondria in the two.

There is another factor, often very confusing, which we have to bear in mind, namely that of the production of coagula in the homogeneous ground substance. They are formed in the protoplasm of the pea radicle by exposure for about 30 minutes to a temperature of 48° C. or higher and also by fixatives containing acetic acid, chromic acid, corrosive sublimate or alcohol. Iron hematoxylin, when imperfectly differentiated with iron alum solution, stains these coagula in the same manner as the mitochondria and in many cases no clearly marked distinction can be made between them, since they both vary very much in their resistance to differentiation. The formation of these coagula is generally accompanied by distortion, fragmentation or destruction of the mitochondria. In the cells of the pancreas these fixatives bring about a distinctly fibrous appearance in the normally homogeneous basophilic substance which is often as resistant to differentiation as are the mitochondria, but the destructive effect is not so apparent as in the cells of the pea radicle.

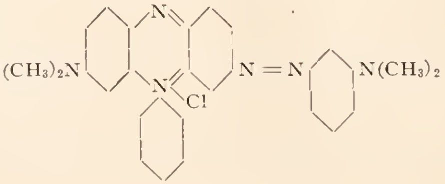
The question of the reactions of plant and animal mitochondria. after fixation, to stains may be dismissed with very few words, When they have been fixed by any method, it is usually possible to color them with any stain, by the Benda method, with fuchsin-methyl-green, with fuchsin-picric acid or with iron hematoxylin, but it is sometimes advisable to select some mordant in order to induce the stain to take. The Benda method is the most difficult and it is sometimes refractory, for no apparent reason, in tissues where the others give excellent results. In other words there is nothing whatsoever specific about the staining reaction.

A number of supravital stains have been discovered for mitochondria in animal cells, of varying degrees of specificity, and it is interesting to note that none of them have heretofore been applied to plant mitochondria. The most delicate is the janus green reaction, which we owe primarily to Michaelis ('99, p. 565). The delicacy of this reaction is shown by E. V. Cowdry's ('16, p. 429) observation that janus green B will stain mitochondria specifically in human lymphocytes in a dilution of half a million (*i. e.*, one part of stain to 500,000 parts of salt solution), and that janus green G will not stain them even in a relatively strong solution.

Janus green B is diethylsafraninazodimethylanilin:



Janus green G is dimethylsafraninazodimethylanilin:



Janus green G differs only in the substitution of a *dimethyl* group in the place of the *diethyl* group in the safranin molecule. Janus blue and janus black I were first introduced as vital stains for mitochondria by E. V. Cowdry ('16, p. 431). Janus blue is the trade name for diethylsafranin-B-napthol and janus black I is a mixture of diethylsafraninazodimethylanilin and some other dye of unknown constitution. Diethylsafranin may easily be made from janus green by splitting off the azodimethylanilin group (Cowdry, '14, p. 269). I have applied these dyes to the mitochondria in the cells of the pea radicle and the acinus cells of the pancreas with the following results.

TABLE III.

Dye.	Cells of Pea Radicle.	Acinus Cells of Pancreas.
Janus green B	+ Intense	+ Intense
Janus green G	−	−
Janus blue	+	+
Janus black I	+	+
Diethylsafranin	?	? + faint
Nilblue B, extra	+ faint	+ faint

It will be seen that the mitochondria in plant cells react to vital stains in precisely the same way as in animal cells, even to this extraordinarily delicate janus green test. It is very much more difficult, however, to get a good stain in plant cells by reason of their tough, cellulose walls offering an almost insurmountable barrier against the penetration of the dye; and janus green is at best but a poor penetrator compared with neutral red, methylene blue and others. For this reason the staining of mitochondria is much slower than in animal cells. Great difficulty was also experienced in finding a suitable medium for the examination of the two tissues. At first they were examined, side by side, in weak aqueous solutions of the dyes to which sodium chloride had been added in varying amounts. This proved entirely satisfactory for the pancreas, but failed with the pea, for which a sugar solution was used. Another trouble with the pea, not encountered with the pancreas, is that the cells are cemented closely together and are therefore so very difficult to separate by teasing, that thin sections of the living pea radicle had to be cut with a razor blade. This method also is objectionable because a comparatively large amount of the tissue juice is liberated which brings about a troublesome precipitate of the dye and makes it necessary to renew it frequently. In short, plant cells are very hard to handle but the stain is none the less specific. The petals of some flowers (narcissus, sweet pea, freesia, etc.) give very much more satisfactory results than the pea since with them the dyes penetrate more easily.

I have also tried to stain the living plants *in toto* by growing them in sand moistened with a strong solution of janus green B. I discovered at once that they react differently to this treatment. Peas were stunted, but grew and flowered without any coloration with the exception of the root hairs and small portions of the epidermis which seemed to be dead. On the other hand, gourds grew vigorously for a time and became intensely stained while growing. They presented a very beautiful appearance because in some of the tissues the dye is reduced to its color base, diethylsafranin, so that the plant is marked off into red and bluish green areas following the distribution of processes of reduction and of oxidation. Cells from the different regions were examined and it was found that the whole protoplasm was stained more or less uniformly and that the mitochondria were not specifically colored. These experiments would seem to indicate that the vital staining of plants with the less toxic azo dyes is full of promise from the point of view of plant physiology.

This specific comparison of the mitochondria in the pea and in the pancreas has shown that morphologically they are almost identical, save for a slight difference in diameter. No forms are present in the one for which counterparts cannot be discovered in the other. If they could be viewed dissociated from their environment it would be next to impossible to tell which belonged to the pea and which to the pancreas. Microchemically they are identical so far as our imperfect methods go. Similar fixatives preserve them, modify them and destroy them in like fashion in both. Even the experimental error is the same. Finally, and most important of all, they react the same way to the janus green test and to other supravital dyes. Accordingly our provisional conclusion can be none other than that the mitochondria in the pea and in the pancreas are composed of precisely the same material.

But this is only a single isolated instance and many facts of interest can be brought to light by a broad general discussion of mitochondria in many forms which I shall now venture to make, based in part upon the literature.

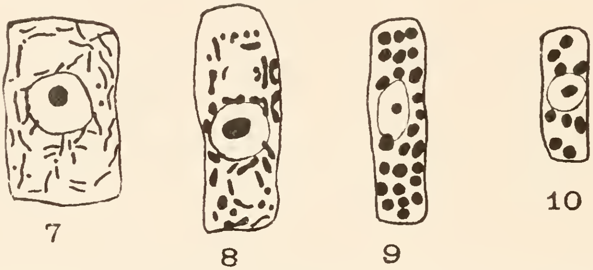
Discussion.

Distribution.

In plants mitochondria have been recorded from the *Angiosperms* to the *Fungi;* but it is difficult or even impossible to demonstrate their existence in the very lowest forms of plant life, like the *Myxomycetes*, the *Schizomycetes* and some of the *Algæ;* though in these groups structures of questionable nature have been discovered which may ultimately prove to be mitochondrial. This absence of typical mitochondria in the lowest plants may be contrasted with their almost universal occurrence in the *Protozoa*. In all forms of animals, from amœba to man, which have been investigated with adequate methods of technique, they occur without exception.

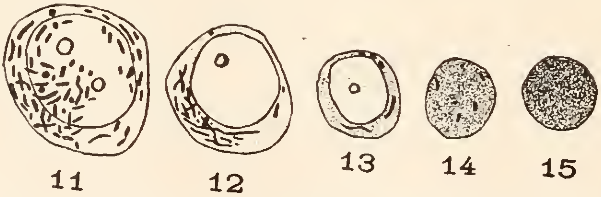
With regard to the different types of cells. In plants, they occur from the tip of the root to the end of the stem, wherever the protoplasm is active, with but few exceptions. The same is true in the various categories of animal cells. They are met with in gland cells, nerve cells and muscle cells; in connective tissue cells, germ cells and almost all others, except in the terminal stages of cytomorphosis. And this is one of the greatest points of similarity between these granulations in the plant and animal kingdoms, that it to say their progressive diminution and final absence in the later stages in the life of the cell.

I refer, for instance, to the decrease in number of mitochondria in plant cells, which runs parallel to the formation of chloroplasts, for it is said (Guilliermond, '12, plates 17–18) that when the plasts are fully mature few if any mitochondria remain (which reminds one of the single large chloroplast and the absence of mitochondria in some algæ); and these are mature and highly differentiated cells. In animals there is a similar disappearance of mitochondria in the life cycle of red blood cells. In the young, nucleated forms they are very abundant, they become less and less so as the cell differentiates; a few persist after the nucleus is lost; but in the fully mature forms, as they occur in the circulation in man, mitochondria are entirely absent. In plants this disappearance is associated with the production of chlorophyll; in animals, with the formation of hemoglobin, two substances with strikingly similar chemical constitution; in both it is progressive and runs parallel with an increase in the degree of differentiation and with the age of the cell, general metabolism being diminished and special functions being accentuated (Figs. 7–15).



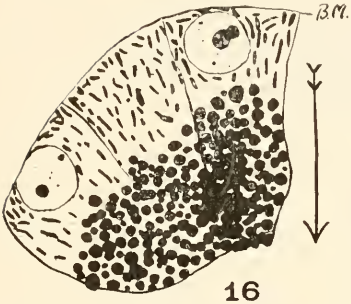
Figs. 7, 8, 9 and 10. Meristem and parenchyma cells of the bean (after Guilliermond, '12, plate 18, modified) showing the progressive disappearance of mitochondria with the formation of plastids containing chlorophyll.

This diminution of mitochondria in cytomorphosis is really of very common occurrence but it attracts attention only in those cells which normally die and are replaced in large numbers, collectively, in the life of the organism.



Figs. 11, 12, 13, 14 and 15. Erythroblast, megaloblast, normoblast and erythrocytes from bone marrow of a rabbit stained vitally with janus green showing the parallelism between the disappearance of mitochondria and the appearance of hemoglobin in the form of a diffuse deposit. (1,500 diameters.)

In the separate regions of the cell, also, there is a general similarity in the distribution of mitochondria in plants and animals. Where the cells are elongated, as in the plerome, filamentous mitochondria are usually distributed parallel to the long axis; which calls to mind the arrangement of mitochondria in gland cells, nerve cells, muscle cells and others. In the serous cells of the parotid they are heaped up in the proximal region next the basement membrane and remote from the lumen (Fig. 16). Where the polarity is reversed as in the thyroid (Fig. 17), the mitochondria are also reversed; while in the epithelial cells of the intestine, they are condensed at both poles (Fig. 18). This, Champy ('12, p. 109) thinks, is indicative of a double polarization for adsorption and for secretion. Corresponding condensations have not been recorded in plant cells.



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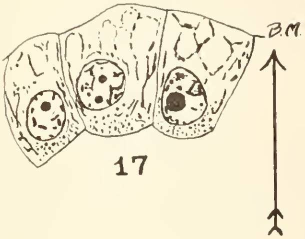

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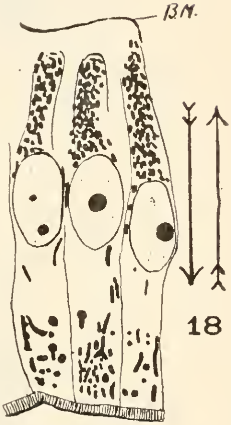

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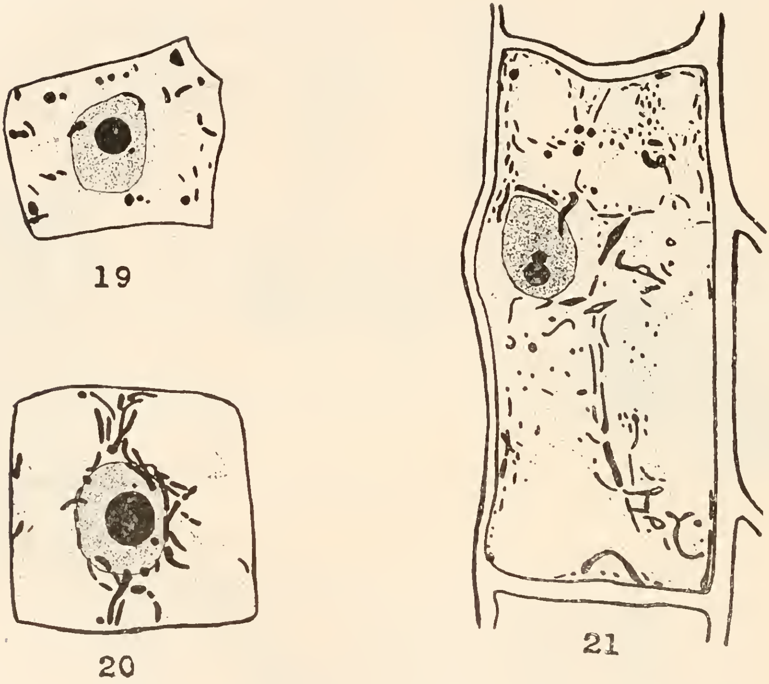
FIG. 16. Serous cells of parotid of mouse with proximo-distal polarity, as represented by the arrow, B. M. being the basement membrane. (1,500 diameters.)

FIG. 17. Thyroid cells (after Bensley, '16, p. 55) with reversed polarity, disto-proximal, in the direction of the basement membrane.

FIG. 18. Intestinal epithelial cells with double polarity, mitochondria being accumulated at both poles. (1,500 diameters.)

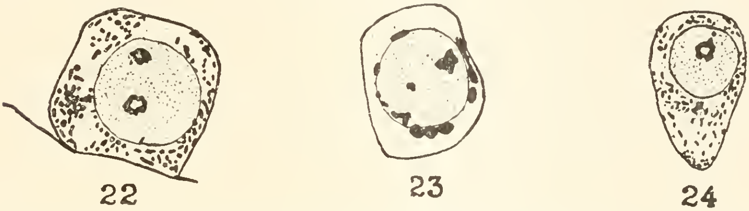
Mitochondria, however, group themselves about the nucleus in both plants and animals. In the early meristem of plants, generally, mitochondria are found indifferently distributed in the protoplasm (Fig. 19). They soon approach and appear to come into actual contact with the nucleus (Fig. 20) in which position they enlarge and form plasts which migrate away from the nucleus and become distributed more or less evenly in the surrounding protoplasm (Fig. 21). Guilliermond has repeatedly described this migration. I find that the mitochondria become progressively more resistant to acetic acid in this process of plast formation. Similarly in the spermatogonia of certain animals the mitochondria are diffusely arranged (Fig. 22); in the spermatocytes they approach the nucleus (Fig. 23) and become so closely applied to it that investigators have been deluded into thinking that they actually originate from it. In the later stages of spermatogenesis they leave the nucleus (Fig. 24) becoming more resistant to acetic acid as Regaud ('08, p. 662) has shown. Indeed the parallelism is very close. Other instances might be cited.



Figs. 19, 20 and 21. Meristem and young and old cortical cells of the pea fixed in formalin and picric acid (Regaud III) and stained with iron hematoxylin showing primary diffuse arrangement of mitochondria, secondary condensation about the nucleus and final even distribution throughout the whole cytoplasm. (1,500 diameters.)

In plant cells one rarely finds a condensation of mitochondria in the peripheral cytoplasm though it is frequently met with in animal cells. It is particularly manifest in certain stages in the growth and maturation of the egg as Van der Stricht ('09, plate 1) and his pupils, among others, have abundantly shown. The causes underlying these strange movements of mitochondria are obscure.

Furthermore, during cell division the mitochondria are distributed in much the same way in plants as in animals. They persist during the whole process; they are absent in the spindle area, whether a definite spindle be formed or not; and they are divided in approximately equal amounts between the two daughter cells.



FIGS. 22, 23 and 24. Spermatogonium, spermatocyte and spermatid from mouse testis fixed in formalin and bichromate (Regaud IV*B*) and stained with iron hematoxylin. Note diffuse arrangement of mitochondria, circumnuclear condensation and final diffuse arrangement. (1,500 diameters.)

In animal cells they are almost invariably disposed in a radial fashion about the centrosome: contrasting strongly with their appearance in *Pustularia* during spore formation when they are entirely absent from the region near the centrosome and are found in a clump in the portion of the cells farthest away from it (Guilliermond, '13*b*, p. 649). No examples of a radial arrangement have to the best of my knowledge been described in the higher plants but this may be due to the well-known absence of a typical centrosome in the *Angiosperms*.

It will be noticed that in animal cells rather more variations obtain in the arrangement of mitochondria than in plant cells, but this seems to be correlated in some way with the fact that animal cells are more generally polarized. I mean polarized for irritability, conduction, secretion, contraction and so forth, properties which do not play so great a rôle in the life of plants, where separate regions of the cell are not so distinctly marked off. Accordingly if one were searching for variations in the distribution of mitochondria in different parts of plant cells one would be inclined to examine cells polarized with reference to light, secretion and so on, where cytoplasmic division of labor may be expected.

Morphology.

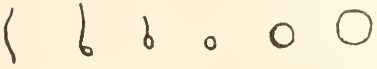
As we pass down the plant scale we find no noteworthy differences in the morphology of mitochondria, even in those *Algæ* which possess them they are alike. Similarly, few animals have mitochondria of distinctive morphology, though in certain species of both plants and animals, either filamentous or granular forms may predominate.

In the different tissues of plants there is some variation in the size and shape of mitochondria. In some cells thick filaments are most abundant, while in other kinds of cells, granular forms of variable size may be found. Networks are rarely met with. The question is complicated by the fact that in certain tissues all morphological transitions between typical mitochondria and true plastids are to be seen; swellings develop in the region of the mitochondrial filaments, which apparently grow larger and larger and ultimately form mature plastids.

In the different tissues of animals there is rather more variation in the morphology of mitochondria. They are usually filamentous in gland cells, rod-like in muscle cells and they are often granular in egg cells. Networks occasionally occur in the pancreas and in spermatogonia as well as in other locations. Even within gland cells of the zymogenic type there is considerable variation. The mitochondria in the acinus cells of the pancreas are uniformly longer and thicker than those in the chief cells of the fundus of the stomach and I find that they are two or three times as long as those in the serous cells of the parotid of the mouse (compare Figs. 2 and 16). The mitochondria in zymogenic cells often possess little enlargements which are supposed to be the precursors of granules of zymogen, and which call to mind the swellings on the mitochondria in plant cells during plastid formation. The similarity of the process may be seen by taking two specific instances; the production of metachromatic corpuscles in plants as figured by

Guilliermond ('13*c*, p. 438) and the formation of fat as described by Dubreuil ('13, p. 104*B*). Compare Figs. 25 and 26.

Striking differences also obtain in the morphology of mitochondria in the different categories of nerve cells. Nicholson ('16, p. 347) has found that they are usually filamentous, especially in the anterior horn cells and in the cells of the reticular formation; they are rod-like and granular in the large and small cells of the Gasserian ganglion, and they occur in the form of large irregular blocks in the cells of the trapezoid nucleus.



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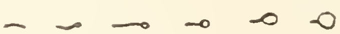

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Fig. 25. Metachromatic corpuscles developing in mitochondrial filaments (after Guilliermond, '13*c*, p. 438).

Fig. 26. Droplets of fat forming in mitochondrial filaments (after Dubreuil, 13, p. 104).

The greater polymorphism of animal cells, with regard to their mitochondrial content, probably results from the fact that their functions are more diversified, for they have a greater variety of duties to perform under different conditions. Apparently their distribution within the cell is more varied in animals than in plants for the same reason (p. 212). The form of mitochondria is apparently independent of the degree of mobility of the surrounding protoplasm.

It is worthy of note that in developing tissues of both plants and animals similar and parallel changes take place in the morphology of mitochondria, at least in certain instances. In the spores of plants and the egg cells of animals, they are usually granular and sometimes filamentous; in the developing tissues of the embryo they are usually filamentous and rarely if ever granular. With the assumption of special functions on the part of the cells in the different organs they change their form. Some remain filamentous and acquire blebs (secreting cells), others become more rod-like (muscle), still others granular, and so on.

A single cell may contain all the types of mitochondria which have been enumerated. This is true for animals as well as for plants. Generally, however, filamentous, rod-like, dumb-bell and granular forms are met with. Filaments, when present, have an astonishingly uniform diameter in the same cell, though they may possess the swellings already mentioned. Segmentation of filaments is of common occurrence in both and it is possible that a coalescence of granules may take place. In some tissues there is considerable variation from cell to cell, while in others the morphology of the mitochondria is quite uniform throughout· Thus the "anthocyane" producing mitochondria, figured by Guilliermond ('13*a*, p. 479), are uniform; those in the cortical cells of the pea radicle, variable in length. In neighboring acinus cells of the pancreas of the mouse they are uniform; in contiguous spinal ganglion cells, variable.

A fundamental distinction may be made between variations in length and variations in breadth, in the case of both plant and animal mitochondria, to which I have seen no reference in the literature. While the mitochondria in the cells of the pea radicle vary greatly in length, filamentous and rod-like forms predominating, their girth is remarkably uniform, throughout the whole tissue, in individual cells and in different parts of the same filament (Fig. 1). The same holds for the pancreas (Fig. 2). The uniformity in girth extends right to the end of the filaments which always end abruptly without tapering. The truth of this statement is made still more apparent by examining any illustration of mitochondria, because artists are wont, quite rightly, to draw each filament with a single sweep of the same pen or brush. The one great exception to this rule is plastid formation in plants and the production of bleb-like swellings in association with secretion in animal cells. The diameter of the mitochondrial filament is fixed, but the length is not; one is stamped on the cell through its organization and is probably dependent on the water content of the surrounding cytoplasm, the other is most likely an expression of growth by accretion affecting the length but not the diameter. We have here two attributes, independently variable, which may perhaps be influenced in different ways, common to both plant and animal mitochondria, which speak more strongly for their identity than any other.

Composition.

We must bear in mind the unsatisfactory nature of the evidence upon which our views of the chemical constitution of mitochondria are based. It is impossible to make a direct chemical analysis of them. We are forced to use solvents, special stains and so forth. We know astonishingly little positively, but we infer a great deal, often on very insufficient grounds. Most of the work has been done with animal cells, and investigators have arrived at the general conclusion that here mitochondria are of the nature of phosphatids, in other words, that they contain fatty acid, phosphoric acid, glycerol and some nitrogen base. Lecithin is an example of a typical phosphatid and has been used to manufacture artificial mitochondria. Briefly the evidence is as follows:

1. In animal cells it has been found that mitochondria are almost completely soluble in alcohol, ether, chloroform and dilute acetic acid. They are rendered insoluble by chromization, and also, in my experience, by treatment with formalin, at least in some cases. They do not stain with Sudan III. or Scharlach R. They are only sometimes blackened with osmic acid.

In plant cells they are also soluble in alcohol, ether, chloroform and dilute acetic acid and they are here rendered insoluble by chromization in exactly the same way. Treatment with formalin also makes the mitochondria insoluble. The whole cell blackens with osmic acid so readily that it is impossible to ascertain just how strongly the mitochondria themselves react to it.

2. It is said, in the literature, that part of the mitochondrial substance, in animal cells, is not soluble in these fat solvents and it has been assumed that this portion is albumin. The results of applying Millon's reagent are uncertain and difficult to interpret.

In view of the statement of Forbes and Keith ('14, p. 73) that vegetable phosphatids differ from those in animal cells in that they nearly always contain sugar firmly bound to the rest of the molecule, we should bear in mind the possibility of sugar being a likely constituent of mitochondria in plants.

3. Artificial mitochondria have been made by Löwschin ('13, p. 203) of lecithin and albumin solutions which apparently

present the same form and solubilities as true mitochondria in both plant and animal cells. They form granules, rods and filaments which multiply by division. He embedded them in glycerin-gelatin, fixed them and found that they stained in the usual way by the various mitochondrial methods.

I have myself experimented with lecithin. I made a very fine emulsion, evaporated it to dryness on glass slides and stained with iron hematoxylin. The bodies which I observed resembled mitochondria in some respects but in others differed so very radically from them that I am unable to confirm Löwschin's observations.

4. The temperature solubility of mitochondria may be also significant because mitochondria, like phosphatids, are thought to have a low dissolving point. Policard ('12, p. 229) observed, in the case of some animal tissues, that the mitochondria are dissolved when subjected to a temperature of from 48° C. to 50° C. in a moist atmosphere from 10 to 30 minutes, while the other parts of the cells remain practically unaffected; and Koch and Voegtlin ('16, p. 59) remark that phosphatids are notoriously unstable to heat. With the hope that this temperature solubility would turn out to be a definite physical test for mitochondria, I experimented with both the pea and the pancreas, observing the precautions outlined on page 199. I found that in both the pea and the pancreas they dissolve at about the same temperature, that is to say, from 48° to 50° C., when treated for 30 minutes. The first change noted is a loss of their filamentous form (see also the Lewises, '15, p. 375); they became granular with indistinct outlines merging into the surrounding cytoplasm and they finally disappeared leaving no trace behind. In the case of the pea, the mitochondrial changes are difficult to make out on account of the production of a confusing coagulation of the ground substance.

5. The specific gravity of mitochondria is generally greater than that of the protoplasm in which they are embedded (Fauré-Fremiet, '13, p. 602). This is determined by the unsatisfactory centrifuge method. When they are thrown down they are said to be of high specific gravity. If the protoplasm is in the physical condition of a gel, rather than a sol, as in the nerve cell, the dis-

tribution of mitochondria is unaltered by centrifuging, as Key discovered. But this is no reason to believe that these mitochondria differ from others in their specific gravity. At any rate, where the method is applicable (*i. e.*, in egg cells) the mitochondria are heavier than protoplasm, in which respect they conform to what we know of phosphatids and differ sharply from oils and neutral fats which rise to the surface and float, instead of being thrown down.

6. In some animal cells mitochondria act as solutes for different substances. Pigments of various kinds are frequently found dissolved in their substance so that they assume the most brilliant hues. Asvadourova ('13, p. 263), following Prenant, calls them "chromochondria" on this account. The presence of droplets of neutral fat within the mitochondrial filaments has also been recorded.

Plant mitochondria behave in exactly the same way, anthocyanin, chlorophyll and other pigments, sugar, starch and even fat being heaped up within them (Guilliermond, '13*b*, p. 647).

7. There seems to be a certain correspondence between variations in the histological picture of mitochondria and variations in the phospholipin content of the same organ on chemical analysis. Thus Mayer, Rathery and Schaeffer ('14, p. 612) have been able to alter the mitochondria experimentally in liver cells. In stages with more mitochondrial substance, chemical analyses show an increase in phosphorized lipoid; in stages with less, diminution.

Unfortunately mitochondria in plant cells have not been investigated from this point of view.

8. And, finally, the very interesting observations of Russo and Rene Van der Stricht must be mentioned. Russo ('12, p. 215) claims to have been able to increase the number of mitochondria in the oöcytes of the fowl by the injection of lecithin and Rene Van der Stricht ('11, p. 435) obtained results which seemed to be confirmatory.

Since there have been no observations along this line in plant cells I have attacked the problem by growing peas in solutions of lecithin. The results were in no sense definite and concise, though the mitochondria did seem to be increased in diameter.

Physiology.

It is generally conceded that mitochondria in plant cells play an important part in the elaboration of chlorophyll and starch. They are thought to do this through the intermediary of the chloroplasts. According to Guilliermond ('12, p. 387) chlorophyll appears in typical mitochondria, increases in amount, other changes take place and the mature chloroplast results. It is not surprising, therefore, that mitochondria have attracted so much attention among European botanists, because the formation of these substances had been under discussion for nearly a century and a deadlock had been reached before these new mitochondrial methods were devised. Indeed the formation indirectly of starch from atmospheric carbon dioxide and water, through the action of sunlight on chlorophyll, is the most fundamental of all vital processes in plants. Mitochondria are concerned in the formation of chlorophyll and thus the very existence of the plant depends on them. Plants furnish the food of animals so that the importance of mitochondria with respect to the food supply is apparent.

Very obviously the mitochondria in typical animal cells can take part in no such process since there are no plastids, and starch is elaborated only in plants. But this is not, as it might seem to be, a fundamental distinction between the two, for many consider the animal mitochondria themselves to be plast-like and to act as such in the elaboration of secretions. The evidence for this, however, is not entirely conclusive and we must bear in mind, in all experimental work, the great importance of the homogeneous ground substance, or environment, in which the mitochondria are embedded. Apparent alterations may occur in the mitochondria which cannot be attributed to the mitochondria themselves, but only to changes in their environment. This is particularly true in the case of their refractive index. The mitochondria may stand out sharply in one cell and be quite invisible in another and yet be identical as far as their function is concerned in the two cells, for variations in the surrounding protoplasm may alone be responsible for the difference in appearance. There are thus two variables, the mitochondria and the protoplasm, either one of which may bring about a visible difference in the living cell independently of the other, though in the majority of the cases they probably act together. So that it is very difficult to tell whether the mitochondria are active or passive agents.

The belief is gaining ground (Kingsbury, '12, p. 46; Mayer, Rathery and Schaeffer, '14, p. 619, and others) that mitochondria are concerned with respiration in animal cells; that is to say, with the taking up of oxygen. This conception is based upon the view that mitochondria chemically resemble phosphatids, it being thought by some that phosphatids outside the body are capable of auto-oxidation. It falls well in line with the very wide distribution of mitochondria as well as with the fundamental nature of the process of respiration. And of course, the same arguments apply to mitochondria in plant cells for plant cells also take up oxygen. It seems that, in this particular, there is no great difference between mitochondria in these two great groups of organisms.

Unfortunately it is not possible to transfer plant mitochondria to animal cells to see what they would do, or vice versa. We are working in the dark, but we have only ourselves to blame because we have not taken full advantage of certain lowly protozoans, like *Euglena viridis* and others, which have, confined in the scope of a single cell, all the properties which we are prone to consider distinctive of both plants and animals. They contain chlorophyll, produce paramylum, a carbohydrate resembling starch, and at the same time engulf, devour and digest certain still more minute organisms. The brief description of mitochondria-like material in *Euglena*, made by Ternetz ('12, p. 463), would serve as a point of departure. The planarian worm, *Convoluta roscoffensis*, which takes in algæ and lives in symbiotic relationship with them would repay further study. We have here an animal containing animal mitochondria with all their attributes which, after symbiotic relationship has been established, "ceases to take in solid food and depends entirely upon its vegetable partners" (Bayliss, '15, p. 295). Another opportunity is afforded by *Chlamydomyxa labyrinthuloides* which when at rest lives as a plant and when active like an animal (Parker and Haswell, '97, p. 49).

One point more. The close resemblance of mitochondria, amounting almost to identity in both plants and animals, may indicate a common ancestral form possessing mitochondria. It is a general assumption that plants antedate animals, and it seems significant that among the lower plants, and not in the lower animals, we find groups in which mitochondria have not been detected. I have particularly in mind the *Algæ*, for in this interesting group some forms contain mitochondria and others do not (M. and Mme. Moreau, '15, p. 730). The absence of mitochondria is interpreted in various ways. Some think that the large chloroplast takes over their function (Guilliermond, '13, p. 86) and it is possible that the mitochondrial substance may be present in an invisible diffuse condition. There is every reason to believe that the possession of formed mitochondria is an attribute even more primitive than the possession of a fully formed nucleus, which, unlike the mitochondria, has gradually assumed a more specialized character. Yeast plants in which the presence of a nucleus is debated (Macallum, '99, p. 67) unquestionably contain them (Janssens and Helsmortel, '13, p. 452), and there are indications that some bacteria also have mitochondrial material. This is beside the question, however, because the *Saccharomycetes* and *Schizomycetes* are probably degraded forms. We can look upon the ancient line of evolution as passing through forms closely resembling some of the *Algæ* of the present day which are devoid of mitochondria, into similar forms possessing mitochondria and which gradually acquired fully developed nuclei in addition. From these nucleated alga-like organisms containing mitochondria, the higher plants and the whole animal kingdom gradually evolved. They stood at the parting of the ways. The geological record, incomplete as it is, seems to indicate that the algæ are the oldest of plants; those of the present day being the lineal and comparatively unaltered descendants of the most ancient ones. It is surprising that mitochondria should persist without great modification through all the storm and stress of millions of years of evolution during which everything else changed except perhaps the primitive living homogeneous ground substance. As they were in the beginning so they are now, on the crest of both animal and plant evolution, their form in *Vaucheria* (Rudolph, '12, Pl. 18, Fig. 9) being equally indistinguishable from that of the oak and of man. This paper shows how much alike they are everywhere. We are tempted to enquire whether the presence of mitochondria in conjunction with a nucleus made evolution possible. This may indeed be true if, as I have already mentioned (p. 223), they are concerned with protoplasmic respiration, which is perhaps the most fundamental of all vital manifestations. Certainly no consideration emphasizes their importance more.

Summary.

This comparison of mitochondria in plant and animal cells brings to light a truly remarkable degree of similarity.

Their *reactions to fixatives, stains and supravital dyes* are almost identical. Similar fixatives preserve them, modify them and destroy them, in like manner, in both. Even the result of experimental errors in the technique is the same. Plant mitochondria react to the janus green test and stain with supravital dyes in substantially the same way as animal mitochondria, though it is certainly more difficult to obtain a good coloration.

Their *distribution* is almost universal. They occur in all plants with the exception of certain *Algæ*, *Bacteria* and *Myxomycetes*, and no animal has yet been discovered which does not contain them. In addition to this, similar and parallel variations occur in their arrangement in the several tissues and even in the individual cells of plants and animals. They must, therefore, be regarded as an integral, perhaps essential, constituent of living matter.

Their *morphology* is identical in plants and in animals. They assume no forms in the one, which are not present in the other. They undergo similar variations in size and shape in different tissues and in different cells in both. If it were possible to view mitochondria dissociated from their environment, it would be impossible to decide whether they came from plant or animal tissues, provided that they did not contain starch, pigment or some other easily recognizable substance, to serve as a clue.

We have every reason to suppose that their chemical *composition* is much the same in both plants and animals, but here our knowledge is for the most part supposition and inference, since direct chemical analyses are obviously out of the question. Their composition, as indicated by solubility with respect to acetic acid, heat and other agents, is certainly subject to similar variations in both.

Their *physiology* is obscure; but their wide occurrence in all protoplasm and their general similarity in all places must mean something. It may mean that, in addition to certain specific functions like the production of chlorophyll, they all have a common duty or part to play in some fundamental vital manifestation like protoplasmic respiration.

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BIOLOGICAL BULLETIN

INHERITABLE MODIFICATION OF THE WATER RELATION IN HIBERNATION OF LEPTINOTARSA DECEM-LINEATA.

WILLIAM LAWRENCE TOWER.

A series of experiments in which *Leptinotarsa decem-lineata* was introduced into the environmental complexes of the deserts at Tucson, Ariz., in nine years of experimentation, have given the results described in this paper; and show how the introduction of an organism from one habitat into another—in these experiments from a mesophytic into desert environment—produce alteration of the water relation in ways that are adaptive in direction and inheritable in character.

The specific result of the experiments concerns the development in the organism, during the period of the experiments, of the capacity to hold water within the tissues so that the intense desiccation of the dry seasons—which are passed in hibernation—does not result in death, and the elimination of the introduced population. The change is adaptive; directly in line with the environmental pressure that is incident upon the population, and in tests is shown to be gametic; in crossing with the normal behaving as a Mendelian dominant; and is not easily reversible.

The materials in the entire series have been pure strains from my laboratory at Chicago; their history, reactions, and genetic composition known for generations before introduction into the Arizona deserts. The materials have at no period in their history been subjected to any experimental operations at Chicago, but are the natural animals, reared under normal conditions, under continuous observation and record. Two strains have been used, No. 99 and No. 100, both derived from nature on the banks of the Calumet River near Chicago, and therefore characteristic of, and in their activities, adjusted to the conditions of the place of origin.

These stocks have two yearly generations: pass the winter in the adult condition, hibernate in the soil, emerge in the spring, as soon as the temperature is high enough; and begin breeding to produce the first summer generation which matures in July; this first summer generation breeds at once, giving the second summer generation maturing in August or early September. These do not breed, but after feeding undergo preparations for, and enter, hibernation during September or early October, where they remain until the arrival of awakening conditions in the following spring.

In nature this reproductive rhythm is never pure nor regular; many of the overwintering population remain alive, or are late in emergence from hibernation, and are able, therefore, to cross with the first summer generation, giving hybrids between two differing conditions in the rhythm of reproduction, and all materials direct from nature are liable to be in one way or another heterozygous with respect to this rhythm. Pure lines, or stocks in which the rhythm of reproduction was homozygous, have been used in these experiments and no others.

The second summer generation does not mature its gametes, and after feeding undergoes changes preparatory to hibernation that are common to insects; the elimination of waste products and the reduction of the water content of the tissues, lowering the freezing point thereof, so that the animals are not killed by the low temperatures of the winter months, an operation common to many plants and animals in resting conditions. Upon emergence in the spring, or for breeding, this water reduction is rapidly compensated for by water derived from the food and that absorbed by the tissues from the atmopshere or hygroscopic water.

The location chosen for the experiments at the Desert Laboratory of the Carnegie Institution of Washington was on the edge of the broad flood plain of the Santa Cruz River at the foot of Tumamoc Hill, a deposit of gravel and boulder beds with a thick surface layer of adobe soil, of uniform texture and composition. All experiments were in cages six feet square and three feet high, with wooden or cement bases that extended into the ground two and one half feet, or below the depth to which the animals burrowed in hibernation. These cages were covered with sixteen mesh pearl wire cloth, which acted to soften the rigors of the desert environment, especially the temperature, rate of evaporation and wind flow, so that the materials while subjected to the rigors of the desert did not receive the whole intensity thereof, but approximately the average conditions.

Throughout the food has been the cultivated potato, as in the original habitat of the materials. All watering was by irrigation about the roots of the plants, and in all respects care was taken to prevent the experiments from having any other than the desert environment about them.

Caged experiments were used instead of isolated locations in the open for two reasons: first, observation and record, as well as protection, could best be maintained in the location and in the arrangements as devised; and second, in caged experiments all selective action by animals that might use these beetles as food was eliminated and the results would, as fully as possible, be those directly due to the physical environmental complex. It developed in the course of the experiments that the arrangements provided not only eliminated this source of complication, but that predaceous enemies, parasites, and epidemics were also absent, no trace of any of them having been discovered, so that as far as I am able to discover, the results are solely the product of the action of the physical environment upon the introduced animals.

The Experiments.

All materials were introduced at the opening of the summer rains, and taken directly from hibernation to Tucson and introduced into the cages, there giving two generations in rapid succession, the second going into hibernation late in the summer, depending upon the environic condition. The following series have given the data for this paper:

T 99, introduced 1908, now in hibernation F_{18}.
T 100, introduced 1909, carried to F_{12} and lost in making repairs.
T 100 A, introduced 1911, now in hibernation in F_{12}.

T 100 B, introduced 1912, now in hibernation in F_{10}.
T 100 C, introduced 1915, now in hibernation in F_4.

Each introduction was taken from the stock at Chicago in the following way: On emergence from hibernation the materials were, of each sex, placed in containers, covered with a cloth, and twenty to twenty-five of each sex removed without inspection, so that there would be no opportunity for selective action on my part, and this parental group of forty to fifty individuals were taken or sent at once to Tucson and placed in the cage prepared with food to receive them. They were then allowed to breed as a population. When the progeny of an introduced group, or if at any other time the population was so large that to breed all was impossible, the same impersonal method of deriving the parental group was resorted to, and it seems highly improbable that with these methods, any selective action could have entered into the series of experiments.

Each introduction has had the same method of derivation from the basal stocks at Chicago, and the same treatment under the conditions of experiment at Tucson, grown as populations, treated as populations, and the results are of the population as a whole, and not of individual lines or mutants.

The series has shown several interesting responses to the introduction into the desert environment, but the most interesting one is the development of a capacity to retain water in the tissues of the second summer, or hibernating generation, instead of eliminating it in preparation for hibernation.

The development of this modification of the animal was first discovered in F_6 of T 99, when a population of over four hundred individuals of the second annual generation was sent to Chicago early in September, 1910 (211 ♂ 234 ♀), placed in a cage in the garden at Chicago, in size and construction like those at Tucson, where they soon entered into hibernation, but absolutely failed to survive the Chicago winter, and were completely eliminated, while a culture of the parent stock in a cage six feet away showed only the normal reaction to that particular winter.

The following year, 1911, tests of this condition were made as follows: T 99 in F_8, of the second annual generation, and T 100 in F_2 of the second annual generation were sent to Chicago early in September and hibernated in the garden alongside of the original stocks, and a further test was made of T 99 in F_7 in which the first summer generation was crossed as pairs with the normal stock at Chicago, and their F_1 allowed to hibernate in the garden along with the normal and the stocks from Tucson. The results that were derived in the following spring are shown in Table I.

TABLE I.

SHOWING THE RESULTS OF THE TESTING OF THE SURVIVAL DURING HIBERNATION IN THE WINTER AT CHICAGO.

Population Generation, Stock.	Test, Winter of	No.	Hibernated.	Emerged.	Per Cent. Eliminated.	
					Before H.	During H.
T 99, F_8	1911–12	100♂	87♂	0♂	13	87
		100♀	90♀	0♀	10	90
T 100, F_2	1911–12	100♂	86♂	5♂	14	80
		100♀	89♀	7♀	11	82
*C 99, F_{20}	1911–12	100♂	91♂	80♂	9	11
		100♀	89♀	84♀	11	16
C 100, F_{12}	1911–12	100♂	92♂	83♂	8	17
		100♀	87♀	79♀	13	8
F_1 Heterozygotes:						
♂ T 99 × ♀ C 99		347♂	317♂	0♂	8.6	91.4
♀ T 99 × ♂ C 99		386♀	342♀	0♀	11.4	98.6

* Chicago normal stock.

These tests were made in wire tubes twelve inches in diameter and forty-eight inches long, placed close together, so that all had as far as one can determine the same conditions during the winter. The findings in the emergence from hibernation in May and June, 1912, show, first, that the condition of non-survival in the T 99 materials concerns the entire population, and that in T 100, the same change is coming over the stock but did not involve the entire population, 6.5 per cent. surviving. These surviving individuals of T 100 were placed in breeding cages, where most of them died, two females and one male only taking part in reproduction, giving a first summer generation of eleven males and fifteen females, which died without breeding, although given every condition for reproduction.

The F_1 heterozygous population showed complete elimination, or a total dominance of the condition present in the Tucson parent, which was also present in an equal intensity in both parents, and equally dominant. On the contrary, the Chicago stocks showed a rather good rate of survival, about the average, and in that the entire test was in a space not over six feet square, with a uniform sandy soil, the result can hardly be attributed to highly localized differences in the winter conditions.

In the summer of 1912 further and more extensive tests were made as follows:

T 99 in F_{10} in the second summer generation to Chicago in September.

T 100 in F_4 in the second summer generation to Chicago in September.

T 100 A in F_2 in the second summer generation to Chicago in September.

The tests for the year comprise the determination of survival in populations that had been at Tucson two, four and ten generations. The results of these tests as determined in the spring of 1913 are given in Table II.

TABLE II.

Population Generation, Stock.	Test, Winter of	No.	Hibernated.	Emerged.	Per Cent. Eliminated.	
					Before H.	During H.
T 99, F_{10}	1912–13	100 ♂	89 ♂	0 ♂	11	89
		100 ♀	92 ♀	0 ♀	8	92
T 100, F_4	1912–13	100 ♂	87 ♂	1 ♂	13	86
		100 ♀	86 ♀	3 ♀	14	83
T 100 A, F_2	1912–13	100 ♂	91 ♂	6 ♂	9	85
		100 ♀	87 ♀	7 ♀	13	80

In T 99 no survivals were found, and the survivals from T 100, although given opportunity to breed, died without issue, and the survivors from T 100 A gave a first summer generation of twenty-six males and thirty-seven females, these a second summer generation of fifty-five males and forty-seven females, of which two males and three females were able to survive hibernation in the following winter, but did not breed after their emergence from hibernation in the spring of 1913.

In the F_1 hybrids in this year (1912), regardless of the direction of the cross, all were eliminated in hibernation, not one appearing in the following spring, again showing the complete dominance of the Tucson trait over the original or normal condition at

Chicago. In the F_2, however, in both crosses survivals occur, and out of 2,137 hibernated, 443 emerged in the spring of 1913. Two random matings were made from these, of twenty males and twenty females, and large populations grown for the second summer generation and hibernated under the usual conditions, gave in the spring of 1914 the following results:

	Hibernated.		Emerged.	
	Males.	Females.	Males.	Females.
Mating *A*	861	905	672	689
Mating *B*	919	945	792	807

These in all ways were not to be distinguished from the normal stocks, and allowing for the normal elimination incident to hibernation under the conditions provided, which averages in my garden about twenty per cent., the experiences of the experiment suggest that we have in the F_2 popuulation of 1912

	Completely Eliminated. 1 DD. + 2 Dr.	Surviving. 1 rr.
Observed	1,694	443
Expected	1,602.75	534.25 if a normal monohybrid.

In that the normal elimination in hibernation of the Chicago stock runs about twenty per cent., the eliminated individuals in this test of F_2 are surely the combined DD and Dr portions of the population, plus the non-distinguishable dead individuals of the (rr) portion of the array.

This method of experimentation and testing has been continued in each year to the date of writing, the results of which show that the condition of non-capacity to survive the winter at Chicago is a gradually increasing product of the populations at Tucson, and that the behavior in crossing of the Tucson characteristic is uniformly that of a Mendelian dominant, the normal Chicago condition appearing in F_2 in numbers that are as close to expectation as could be expected.

Since the discovery of this condition in T 99, all of the introductions of *L. decem-lineata* have been tested in the hibernating generation with respect to their capacity to survive the winter conditions of the habitat from which the stock came. The results of these tests are presented in Tables III., IV., V. and VI.

Table III.

Showing in Culture T 100, the Complete Failure of Survival in the Populations of This Culture after Six Generations in the Deserts of Tucson.

Population Generation.	Test Winter of	Number Taken.	Hibernated.	Emerged.	Per Cent. Eliminated.	
					Before H.	During H.
F_6	1911–12	100 ♂	87 ♂	0 ♂	13	87
		100 ♀	90 ♀	0 ♀	10	90
F_8	1912–13	50 ♂	47 ♂	0 ♂	6	94
		50 ♀	44 ♀	0 ♀	12	88
F_{10}	1913–14	25 ♂	23 ♂	0 ♂	8	92
		25 ♀	22 ♀	0 ♀	12	88
F_{12}	1914–15	50 ♂	44 ♂	0 ♂	12	88
		40 ♀	39 ♀	0 ♀	22	78

Table IV.

Showing the Results of the Tests in Culture T 100 A, in which Elimination During Hibernation is Shown to Increase Progressively, Ending in a Condition in which Survival of the Winter at Chicago is no Longer Possible for the Populations in this Culture.

Population Generation.	Test Winter of	Number Taken.	Hibernated.	Emerged.	Per Cent. Eliminated.	
					Before H.	During H.
F_2	1911–12	100 ♂	91 ♀	6 ♂	9	85
		100 ♀	87 ♀	7 ♀	13	80
F_4	1912–13	100 ♂	84 ♂	1 ♂	16	83
		100 ♀	87 ♀	3 ♀	13	84
F_6	1913–14	50 ♂	44 ♂	0 ♂	12	88
		50 ♀	45 ♀	0 ♀	10	90
F_8	1914–15	100 ♂	89 ♂	0 ♂	11	89
		100 ♀	93 ♀	0 ♀	7	93

Table V.

Showing the Survival Tests made in the Second, Fourth, Sixth and Eighth Generations of the Culture T 100 B, in which the Progressive Increase in the Elimination During Hibernation from the Second to the Eighth Generation, Results in Complete Elimination in the Last Two Tests Made in this Series.

Population Generation.	Test Winter of	Number Taken.	Hibernated.	Emerged.	Per Cent. Eliminated.	
					Before H.	During H.
F_2	1912–13	100 ♂	89 ♂	5 ♂	11	84
		100 ♀	86 ♀	7 ♀	14	79
F_4	1913–14	100 ♂	91 ♂	2 ♂	9	89
		100 ♀	90 ♀	3 ♀	10	87
F_6	1914–15	100 ♂	86 ♂	0 ♂	14	86
		100 ♀	87 ♀	1 ♀ died	13	86
F_8	1915–16	50 ♂	45 ♂	0 ♂	10	90
		50 ♀	42 ♀	0 ♀	16	84

TABLE VI.

SHOWING THE TEST MADE UPON CULTURE T 100 C, INTRODUCED INTO THE TUCSON DESERTS IN JUNE, 1915, WHERE IT REPRODUCED, THE SAMPLE OF THE SECOND SUMMER GENERATION, TAKEN TO CHICAGO, IN SEPTEMBER, AND SUBJECTED TO THE SURVIVAL TEST IN THE WINTER OF 1915–16.

Population Generation.	Test Winter of	Number Taken.	Hibernated.	Emerged.	Per Cent. Eliminated.	
					Before H.	During H.
F_2	1915–16	50♂ 59♀	46♂ 44♀	4♂ 6♀	8 12	84 76

These tests shown in the above tables have been made in the wire tubes, placed close together, and having as far as could be determined the same conditions during a given winter. Undoubtedly the testing in tubes favors the culture in the elimination of possible enemies in the soil, and in fact, the survival of the normal stocks in the tubes at Chicago is higher than in large open cages, so that any action of the tube is favorable to survival rather than otherwise.

In all of the introductions precisely the same action is going on, and in all of the tests the same result shows at about the same rate of progression, namely, that the capacity to survive the winter diminishes progressively as the number of generations increases in which the stocks have lived under the conditions of the Arizona deserts. The greatest change comes between introduction and the hibernation of the first year.

At Chicago for some years I have made routine tests of certain of the activities concerned in hibernation, in that this period of passing of the unfavorable portion of the year in their habitat is a season of possible elimination, of possible selective action, therefore, one of possible effectiveness in the evolution of the population. In Table VII. I have shown a series of tests made in the stock C 100 from the eighth to the eighteenth generations, inclusive. These were all made in wire tubes sunk in the ground, under similar conditions of soil, moisture and climatic exposure.

The tests in Table VII. show that in the preparation for hibernation, on the average, eleven per cent., of the tested materials are not able to complete the preparations and do not enter into hibernation. These will be found dead upon the surface of

Table VII.

Showing in the Original Culture of *L. decem-lineata* No. 100, at Chicago. Tests Made to Determine the Elimination in the Hibernating Population, in Preparation for Hibernation, the Number that Hibernated, and the Percentage Eliminated During Hibernation, with the Percentage of Survivals in the Following Spring.

Stock Culture No. 100, Chicago.

Population Generation.	Test Winter of	Number Taken	Hibernated.	Emerged.	Per Cent. Eliminated.	
					Before H.	During H.
F_8	1910–11	100♂	91♂	83♂	9	8
		100♀	87♀	80♀	13	7
F_{10}	1911–12	100♂	96♂	82♂	4	14
		100♀	83♀	79♀	17	4
F_{12}	1912–13	100♂	94♂	80♂	6	14
		100♀	88♀	77♀	12	11
F_{14}	1913–14	200♂	181♂	160♂	9.5	10.5
		200♀	173♀	164♀	13.5	5
F_{16}	1914–15	100♂	88♂	71♂	12	7
		100♀	90♀	67♀	10	13
F_{18}	1915–16	100♂	83♂	77♂	17	6
		100♀	91♀	74♀	9	15
Totals		700♂	633♂	553♂	av. 9.583+	av. 9.916+
		700♀	612♀	541♀	av. 12.416+	av. 9.166+
		1,400	1,245	1,094	av. 11	av. 9.541+
Total elimination in connection with hibernation, av.					20.54+	

the ground in the tube, but what the cause of the elimination is has not been determined, and may possibly not be possible of determination as the population will not breed until after hibernation and it is therefore impossible to attempt to get a culture of these individuals for further testing, and further there are no indications that have been detected that they are in any way different from the remainder of the tested population. Moreover the sample for testing has always been drawn from the stock culture, in an absolutely impersonal manner and at random so that the elimination may for the present be regarded as a chance elimination in the preparations for hibernation, of about the same value in each hibernating generation, therefore of no probable modifying action upon the population.

From the same table it appears that during hibernation there is on the average an elimination of nine and one half per cent.

of the tested populations, or a total elimination incident to hibernation of the second summer generation of these animals of about twenty per cent. of the population, about eighty per cent. emerging in the following spring. In nature, however, the elimination is greater, due no doubt to agencies that are eliminated under the conditions of these tests, which completely cut off all the losses that might come from burrowing animals that might use them as food, and probably other agencies as well.

In Table VIII. are given the combined results of the testing of the introduced cultures at Tucson, respecting their ability to survive the hibernating season of their native habitat.

TABLE VIII.

SHOWING THE COMBINED RESULTS OF THE TESTING OF THE CULTURES T 99, T 100, T 100 A, T 100 B, T 100 C, FROM THE SECOND TO AND INCLUSIVE OF THE SIXTEENTH GENERATIONS. IT SHOULD BE NOTED THAT THE PERCENTAGE OF ELIMINATION BEFORE HIBERNATION, REMAINS ABOUT AT THE AVERAGE FOR THE SPECIES AS SHOWN IN TABLE BUT THAT THE ELIMINATION DURING HIBERNATION INCREASES, UNTIL ABOUT THE SIXTH GENERATION, WHEN IT BECOMES TOTAL, AND REMAINS SO TO THE END OF THE SERIES TO DATE.

Population.		No.	Hibernated.	Emerged.	Per Cent. Eliminated.		Per Cent. of Population Surviving.
					Before Hib.	During Hib.	
F_2	T 100 A.... T 100 B.... T 100 C....	250♂ 250♀	226♂ 217♀	15♂ 20♀	9.33+ 12.66+	84.33+ 78.66+	7.5
F_4	T 1100 A.... T 100 B....	200♂ 200♀	175♂ 177♀	3♂ 6♀	12.5 11.5	86 85.5	2.25
F_6	T 100...... T 100 A.... T 100 B....	250♂ 250♀	217♂ 222♀	9♂ 1♀ died	13 11	87 88.66+	.20
F_8	T 99...... T 100...... T 100 A.... T 100 B....	300♂ 300♀	268♂ 270♀	0♂ 0♀	10 11	90 89	0
F_{10}	T 99...... T 100......	125♂ 125♀	112♂ 114♀	0♂ 0♀	9.5 10	90.5 90	0
F_{12}	T 99...... T 100......	200♂ 200♀	177♂ 178♀	0♂ 0♀	12 11	88 89	0
F_{14}	T 99......	100♂ 100♀	89♂ 93♀	0♂ 0♀	11 7	89 93	0
F_{16}	T 99......	100♂ 100♀	86♂ 90♀	0♂ 0♀	14 10	86 90	0

Comparison of the data in Tables VII. and VIII. shows conclusively: First, that the elimination in the preparation for hibernation remains at about a constant figure in both the introduced materials at Tucson and in the original materials that have lived continuously at Chicago; second, that the elimination during hibernation takes a great increase in all the second generations tested, from 9.5 per cent. to over 80 per cent., and then in the next following years increases slowly until the sixth, when elimination is complete and continues to be so, no capacity to resist the Chicago winter conditions appearing in any of the Tucson materials in the later generations.

Beyond question, therefore, some change has taken place as a consequence of the introduction of *L. decem-lineata* from the habitat at Chicago into the deserts at Tucson, in the change from a moist habitat into one of intense desiccation during the resting season, which is passed in hibernation. This change is made graphic in Fig. 1, showing the line of elimination in the preparation for hibernation, remaining constant throughout the

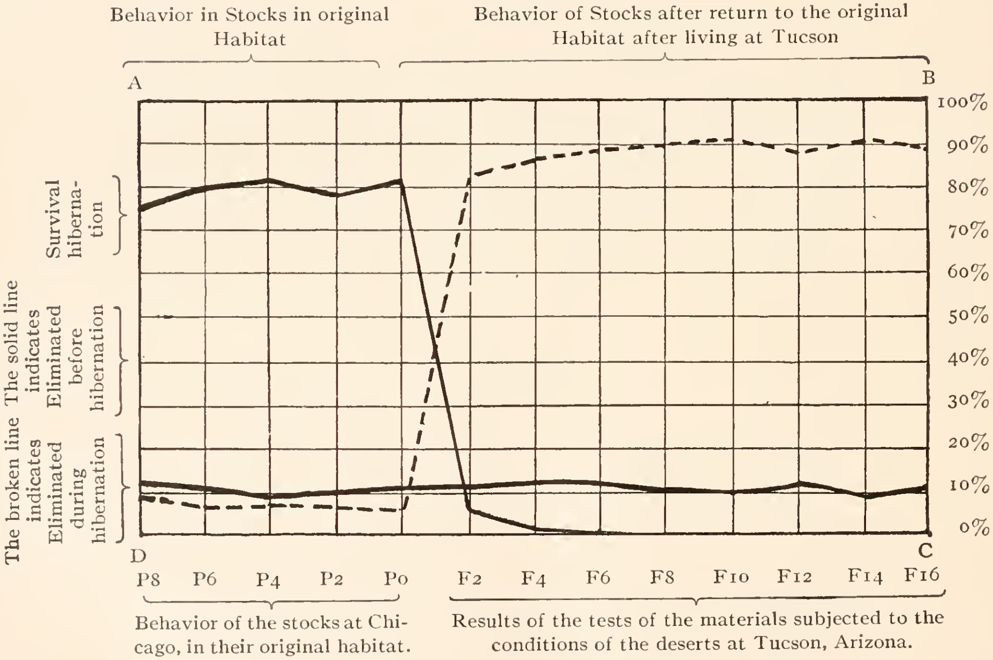

FIG. 1. Graph showing the curve of survivals from hibernation, of elimination in preparation for hibernation, and of elimination during hibernation.

series, the sharp changes in the line of survival of hibernation and in elimination during hibernation that occur between the introduction and the first hibernating period, at the end of the second generation.

In hibernation temperature and humidity in the soil are the two chief factors concerned with these animals, and their action upon the organisms is difficult of isolation. In the two environments chosen for the experiments, the conspicuous difference is one of humidity, not of temperature; soil conditions at Chicago during the period of hibernation having a high and rather constant water content, whereas at Tucson the relation is one of continuously diminishing humidity. At the close of the summer rains at Tucson, the intense desiccation accompanying the high temperatures of the dry after summer, rapidly lowers the water content in the soils until the onset of the winter rains in December, and again in the dry fore summer from the end of the winter rains in March to the beginning of the summer rains in July, the water content is progressively lowered until at the end of the period it may be as low as 5 per cent. or even less in the upper layers of the soil. It is this latter period in which the most intensive elimination of the cultures at Tucson takes place.

In desert organisms, in general, some mechanism is present by which the water content of the resting organisms is conserved during the dry and resting periods in the year, otherwise the plants or animals are not able to survive the hostile conditions of these rigorous portions of the year that are so characteristic of every desert environmental complex, and which may be prolonged, irregular, and highly variable in their manifestation in different years. Consequently, surviving desert types must meet, not only the average extreme and hostile conditions in their existence, but irregular and extreme manifestations of the environic complexes, often of prolonged duration. In other words, a desert environment is not only a most rigorous one, but also about the most variable habitat that an organism has to meet.

The response found in these experiments at Tucson suggests at once that the alteration is one of water relations, one in which the capacity to hold the water in the hibernating generation has developed. Tests of the capacity to hold the water in the tissues against desiccation have been made in the different series of experiments, with rather striking results.

These tests are made by taking about equal weights of one of the Tucson cultures from the generation that is hibernating, and from the cultures at Chicago, enclosing them in small wire cloth containers, the two, then subjected to intense desiccation by the passage of a current of dehydrated air at a uniform rate through the materials until death has resulted, and the materials attained a uniform dry weight, weighings being taken every twelve hours.

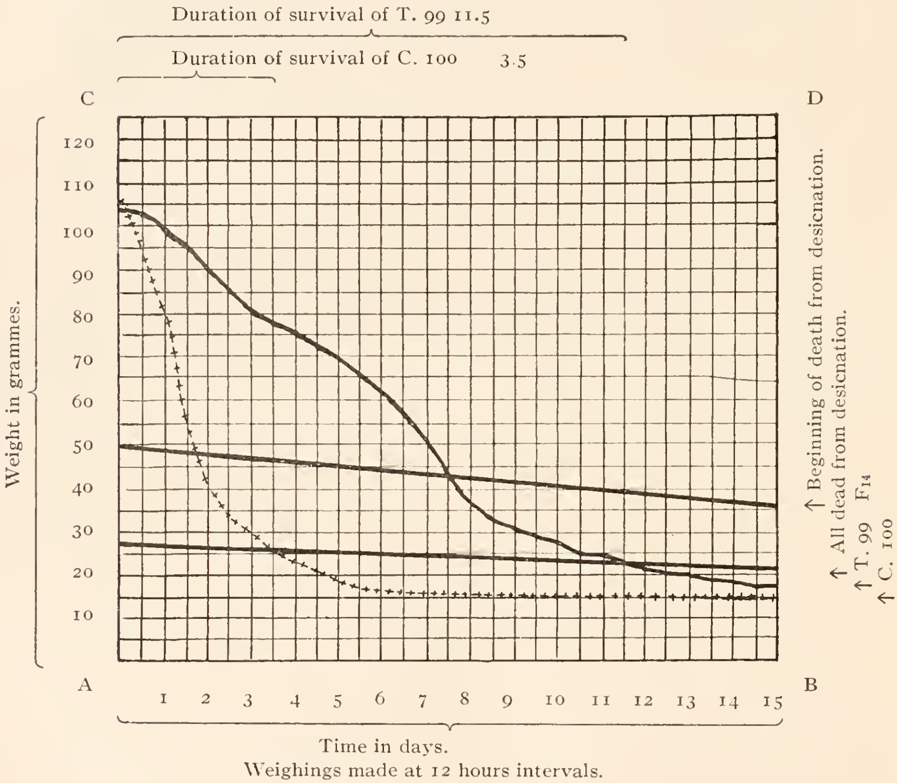

FIG. 2.

These tests show that the Tucson materials, especially after the sixth generation at Tucson, have constantly the capacity to hold onto the water content in their tissues, are more resistant to desiccation, and in every way are different in their reactions to the water loss than are the same materials that have lived at Chicago continuously. In Fig. 2 I have plotted the results of one of these dual tests made upon T 99 in the fourteenth generation of its existence at Tucson, and the stock C 100 from Chicago.

The curve of response of the materials at Chicago shows a rapid water loss in the first day, the onset of death from desiccation by the end of the second, and the death of all in this test at three and one half days, with the attainment of a constant dry weight under the conditions at about the sixth day. In the sample from Tucson, however, the curve of response to desiccation shows a much less rapid loss, the first deaths not occurring until the seventh day and total death not until the twelfth day, and dry weight constancy about the fifteenth, but not as low as in the culture of Chicago materials. The temperature in this test was constantly 20° C., the temperature used in all such testings.

There is a very obvious difference in the ability of the two sets of organisms to let go of the water in the tissues under-desiccating conditions in the environment. In these tests the conditions were most severe, with absolutely dry and rapid passage of air, in all respects immensely more severe than are ever obtained in any desert complex during hibernation, as the animals are then protected in the ground and desiccation is slow and gradual. However, the desert adjusted group shows that it has the ability to hold the water, or less capacity to let the water loose from its tissues when subjected to the pull of an intensely dry environic condition, and this one difference would decide the fate of either set of materials in different environments.

The eliminations in the survival tests made at Chicago of the samples from the Tucson stocks, are clearly due to their not being able to let go of the water in their tissues with sufficient rapidity to keep the freezing point below the temperature of the soil, consequently they are actually frozen by the decreasing temperatures accompanying the onset of the northern winter, and examination of test cultures made for the purpose at Chicago shows that few of the individuals in the test survive beyond December first, and none have been found alive by the end of the same month. Further many of the individuals seen in these examinations when recovered from the ground show frost crystals within the tissues, and extensive disruptive actions thereof.

Repeated tests in the different cultures at Tucson, at early and late generations during their history, show that the retention of water in the tissues against the influences of a strongly desiccating environic complex gives a series of findings that are in close accord with the data derived from the survival tests of the same materials at Chicago; a sharp change in this capacity between introduction and the first period of hibernation, and thereafter a slower increase through the following hibernation periods, until the full capacity is developed, at about the sixth generation after introduction.

From all the different aspects in which this response to the changed environic complex is viewed, it gives only the one conclusion, that there has resulted from the sharp change in the living conditions the development of a capacity to hold water and not lose water in the presence of conditions in the surroundings, which in the original materials causes rapid loss of water with eventual death. The modification is, therefore, of a direct adaptive nature, in direction and kind to meet the needs of the new environment.

What the alteration has actually been in the mechanism of the organism, I do not know. Thus far I have not been able to detect any anatomical modifications, or changes of a cytological character that have taken place. Water loss in these animals is through the dermal glandular secretions and respiratory activity. As far as I can determine, there is no decrease in the dermal glands that are present, either in size or number, or any alteration in their distribution, although I have searched diligently therefor. Moreover, there are no indications of a thicker cell wall, either in the hypodermis or of any increased thickness in the cuticular linings of the tracheal tubes.

Whether it is due to changes in the permeability of the cell membranes or to changes in the colloidal contents of the cells is a matter of opinion, as I have no evidence that supports either view with any certainty, and accurate determination of either of these is difficult, if not quite impossible. Regardless of what the actual change may consist of, it is an evolutionary modification, that is directly adaptive in response to altered conditions of life, and as such is well worth careful examination and further investigation.

Perchance it may seem that it is easy to arrive at results of this kind. To prevent any such misapprehension, the experiences in the introduction of other species into the same environic complexes may be cited. During the years in which these experiments with *L. decem-lineata* have been in progress, there have been introduced with the same care and persistency *L. signaticollis*, *diversa*, *undecimlineata*, *panamensis*, *multitæniata*, *oblongata*, *haldemani*, *juncta*, and *dilecta*, representing a diversity of environmental origins and adjustments to temperature and moisture during the hibernation period, and in none of them has anything like the result described in *decem-lineata* been found. The closest approximations thereto are in the species *multitæniata*, *oblongata*, and *signaticollis*, all of which live in haibtats having rather high rates of desiccation during the resting season, and survival in the case of these introductions has been irregular, and the product of favorable conditions in the particular winter, rather than any alteration resulting from the introduction. All of these species, further, live in areas in which the temperature in the resting season is fairly high, so that there is no necessity for the reduction of the water content to prevent elimination by frost action.

INHERITANCE.

It was shown in connection with some of the earlier tests that the alteration when crossed with the normal condition at Chicago, gave an F_1 that was completely eliminated during the test of survival of the winter, and that F_2 showed a result suggesting the survival of the recessive extractives of the normal type. Further crossings of these have confirmed this original finding and extended the information as to the behavior of the alteration in inheritance, thus showing that the change between the conditions of the populations at Chicago and Tucson is of a gametic nature, and not somatic or temporary.

All tests of the heritability of this modification have been made with the sixth or later generations, for the reason that the alteration was apparently not at its full development until then, and in that the only means of distinguishing between the two conditions has been the survival test under the winter conditions at Chicago, earlier generations in which there were survivals of this test, would have given only results that had an error in them not possible of estimation. After the sixth generation at Tucson, all the cultures have thus far given the uniform result in crossings with the normal at Chicago, regardless of the direction of the cross, and these summated results are given in Table IX.

Table IX.

F_1 Populations.	Pairs.	Population.	Total Tested.	Hibernated.	Emerged.	% Eliminated.		Survived.
						Before.	During.	
Pairs T. 99 × C. 100 C. 100 × T. 100 T. 99 × C. 99 C. 99 × T. 99	42	7	2,987	2,651	0	11.25 ±	88.75 ±	0
F_2 population	65	11	6,411	5,814	1,573	9.3 ±	66.1 ±	24.4 ±

In making these tests of the heritability of the alteration in the Tucson cultures, the F_1 series were all from matings of the first summer generation, whose progeny would hibernate, and for the F_2 tests, the over-wintering generations at Tucson and Chicago were crossed at Chicago, and the second summer generation tested. The results show in all that the F_1 population are in no instance able to survive the test, that the condition of the Tucson cultures is dominant, but that there is a segregation in F_2 and a survival from the test that is for the total series 24.4 per cent., about the expected proportion of the extracted recessives. These in breeding in F_3 show no indication of the Tucson condition, either in pairs or mass cultures, all surviving the hibernation test in F_4 in all respects like the normal Chicago materials.

The behavior in inheritance shows, therefore, no points of interest aside from the fact of its heritability, and the fact that a condition that has arisen rapidly, but apparently progressively, behaves in crossing when fully established as a Mendelian dominant, but its behavior in a Mendelian way is no indication of its origin after its production.

NATURE OF THE ALTERATION.

As already shown, the alteration in the Tucson cultures is physiological, involving a specific aspect of the reproductive cycle, that of the resting period which is passed in hibernation, and the only sure test of its existence is that of the survival under the conditions of the northern winter, at the place of origin of the stocks. This fact limits the possibilities of testing and experiments with the modifications that are possible, since no certain structural alterations in correlation with this change are demonstrated up to the present, and the differences in the color in the materials are too insecure criteria to be used at present.

What the physical or chemical changes are there is no indication at the present time, and one interpretation has about the same chances of being the correct one as any other excepting for the fact that the modified materials show a difficulty or slowness in water loss which seems to me to suggest more probably some altered condition in the colloids.

The chief question that concerns us in the consideration of the nature of the alteration, is whether it is an added or new condition, or the revival of an ancestral one.

The species *L. decem-lineata*, originally confined to the eastern slopes of the Rocky Mountain uplift, and eastward over the Great Plains into western Kansas and Nebraska, from whence it gradually spread eastward to the Atlantic coast, has as its nearest relatives, a series of species that are confined to the high plateaus of Mexico; areas of semi-desert with arid conditions, especially in the resting season. Using the usual criteria of kinship, similarity in structures and habits, the species *L. multitæniata* and *oblongata* are the nearest relations of this northern species phylogenetically.

All three species interbreed rather freely, are in developmental sequences similar, as in their ecological relations, in their reproductive cycles, in relation to the growing season of the habitat in which they live. These relations and conditions I have recorded in a previous publication, along with the probable relations and phyletic history and relations of these organisms.

Both of the southern species in all of the locations in which I have information concerning them, must encounter during their period of hibernation desiccation in differing degrees of intensity, from a rather low one on the Plateau of Ahnuac, to intense conditions in the deserts of San Luis Potosi, and in hibernation do not go deep in the soils, but remain in the upper layers, or near the surface, whereas *decem-lineata* in its range goes deep in the soil. Owing to the disturbed and impossible conditions in Mexico, since this condition was discovered in the Tucson cultures, it has been impossible to either obtain new materials from the Mexican locations, or make desirable observations and tests that might have been made had more happy conditions prevailed. Fortunately some experiments made in 1908 and 1909 for other purposes have given data as a by-product that is of interest in this connection.

In both of these years crosses were made in the garden at Chicago of the C 100 stock and freshly obtained materials of both *L. oblongata* and *L. multitæniata*, the latter from Chapultepec near the City of Mexico, the former from Cuernavaca in the State of Morelos, Mexico. These were of the first summer generation in all species, and the F_1 populations were hibernated in the garden at Chicago under the full intensity of the winters of 1908–9 and 1909–10, with the results that are shown in Table X., all the materials being from the mating of pairs, and not from population cultures.

The experiences in the crossing of the three species shown in Table X. with the elimination of the hibernation F_1 population during the winter months is highly suggestive, and would give the basis for an interesting series of tests were it possible to obtain fresh materials from the Mexican locations. The complete dominance of the Mexican species over *decem-lineata* in the matter of survival in hibernation, indicates that in them the same or essentially the same condition exists as in the cultures at Tucson. Unfortunately, I have not had the necessary materials for the testing of their resistance to desiccation, and can therefore only conclude that the close identity in the survivals from hibernation is due to the same causes as it is in the tests of the Tucson cultures. Unfortunately, I have no data concerning the survival of the Mexican types at Chicago that are at all comparable, so that I am not sure whether the pure Mexican types are able to survive the Chicago winter or not, but it is my opinion that they would not.

TABLE X.

		Pairs.	F1.		Hibernated.		Emerged.	
L. 10 lin. C. 100 ♂ × ♀ L. mult Chap.*	1908–09	7	170 ♂	196 ♀	147 ♂	171 ♀	0 ♂	1 ♀ died
	1909–10	14	278 ♂	304 ♀	229 ♂	258 ♀	2 ♂ died	1 ♀ died
“ ♀ × ♂ “	1908–09	6	145 ♂	144 ♀	119 ♂	123 ♀	0 ♂	0 ♀
	1909–10	10	309 ♂	301 ♀	267 ♂	271 ♀	1 ♂ died	0 ♀
			902 ♂	945 ♀	762 ♂	823 ♀	3 ♂ died	2 ♀ died
L. 10 lin. C. 100 ♂ × ♀ L. obl. Cuer.†	1908–9	4	96 ♂	104 ♀	74 ♂	91 ♀	0 ♂	0 ♀
	1909–10	6	201 ♂	197 ♀	161 ♂	174 ♀	0 ♂	0 ♀
“ ♀ × ♂ “	1908–9	5	131 ♂	127 ♀	100 ♂	93 ♀	0 ♂	0 ♀
	1909–10	9	246 ♂	265 ♀	197 ♂	213 ♀	0 ♂	0 ♀
			674 ♂	693 ♀	532 ♂	571 ♀	0 ♂	0 ♀
L. obl. Cuer. ♂ × ♀ L. mult. Chap.	1908–9	2	77 ♂	79 ♀	64 ♂	60 ♀	0 ♂	0 ♀
	1909–10	7	193 ♂	201 ♀	154 ♂	183 ♀	0 ♂	0 ♀
“ ♀ × ♂ “	1908–9	3	66 ♂	73 ♀	60 ♂	64 ♀	0 ♂	0 ♀
	1909–10	4	107 ♂	119 ♀	91 ♂	99 ♀	0 ♂	0 ♀
			443 ♂	472 ♀	369 ♂	406 ♀	0 ♂	0 ♀

* Chapultepec. † Cuernavaca.

The two Mexican species introduced into the deserts at Tucson showed in the first introductions in 1908 a huge elimination during hibernation, but they survived in the following year and continued a precarious existence for several years thereafter. Later introductions survived, or failed, for reasons not associated with the behavior in hibernation.

Upon the basis of the probable phylogenetic relations, the facts of the developmental cycles and the similarity in the ecological relations and needs, the data in the crossing of the species under the Chicago conditions and the survival values of the F_1 found therein, and the obvious recessiveness of the northern species in its hibernation behavior to the southern, gives the basis for several conclusions of interest as to the nature of the change.

(1) Assuming that the phylogenetic picture that we have drawn of the three species is essentially correct, and that *L. decem-lineata* is the younger derivative from the Mexican types, an essentially fair conclusion as far as conclusions in this line are at all possible, then the results of the tests show that there is a dominance of the older phylogenetic condition over the newer; or

(2) It may be concluded that in the origination of *L. decem-lineata* in its northern range, there had been a loss of an agent in the organization, which when present, produced retention and slow loss of water under desiccation, and its absence ease of loss, the experimental tests showing, therefore, the dominance of the presence over the absence. In that the reaction in crossing is a monohybrid one, one unit of difference may be assumed; or

(3) It may be concluded that we are dealing with a physical state in the living mechanism that exists as two conditions, alternative to one another in crossing reactions, but, which by the pressure of the medium may be altered to present first one aspect of its reaction, and under other conditions of the medium, the opposite aspect of its effect in the reactions of the living mechanism.

Which of the three possibilities is chosen as the correct one will depend entirely upon one's philosophical background, and from each a rather entertaining and plausible argument can be made. From purely mechanistic and physico-chemical considerations of living phenomena, I am inclined to the third possibility, as not only the more probable and thinkable, but also the one that as a working hypothesis offers the only opening for further experimental investigation.

In this preference I am no doubt prejudiced in my choice by many experiences with the behavior of the non-hibernating generation in which the behavior is easily, rapidly and positively altered, reversed at will by changes in the water content of the medium, producing reversibility of many of the trophisms of that generation. However, in this first summer generation, another condition of the reproductive cycle is under observation, and in that the reproductive cycle itself is present in two sharply alternative conditions, to breed or not to breed, activity or hibernation, and further since these states are known in experiments to be alternative in hereditary behavior, one must not be too fast nor certain in arriving at conclusions in these complicated physiological activities.

Regardless of what one may believe concerning the alteration or the findings of subsequent investigations, certain aspects of its nature are demonstrated, its sharp alternativeness in heredity, its rôle in the determination of survival in different habitats, its directly adaptive nature; and its alteration in direct response to the conditions of the medium in adaptation to those conditions; these aspects of its nature show an alteration, a character capable of alteration, in ways that are of importance in the survival of the organisms, and that might be of decided value in evolutionary activities, speciation, and in ecological and distributional relations and results.

Method of Origin.

This account deals with experiments that are concerned with populations, and the results seem to be those of the alteration of the population as a whole. As shown, the experimental method was such that as far as the operator was aware, the choice of materials was random and entirely impersonal, and throughout any personal influence has been eliminated as far as known. Further, the living of the cultures in cages eliminated the action, if any, of enemies and further gave the cultures better conditions of life than they would have had in nature. Insofar as is known, therefore, the series deals with the response of an introduced population to the physical conditions of its environment. What are the methods of origin of this response and alteration? There are several possibilities:

1. That the original population consists of different pure lines, which under the conditions of the original habitat interbreed, and are hidden from recognition, but that under the Tucson conditions one has been able to survive with the characteristics found, and that the progressive development of its full intensity is the gradual elimination under the conditions of the experiment, of all but the one line with the characteristics finally developed. The conditions of experiment are, therefore, acting only as a sieve to separate out those capable of survival from those not

capable thereof, and we are determining in the experiment only some aspects of the composition of the population of the species in its original habitat.

2. That the introduced populations are uniform in composition, but that as the result of the altered conditions of life, variations are induced, of which only those whose reactions enable them to resist desiccation have survived; a natural selection of the fit by the physical environic factors.

3. That the population has latent within it the factor for water retention, and that this latency from an ancestral conditions under the conditions of the new habitat, is reactivated, mutates to the ancestral state in the materials of the experiments.

4. That the water relation in connection with hibernation is one due to some physico-chemical relation in the living colloids, that exists in one of two aspects, a condition of the substance, its type of manifestation depending upon the nature of the conditions of the medium.

There may be other possible methods whereby the condition is produced in the experiments, but these seem to me the most obvious possibilities.

That the results are due to the isolation under experiment from the introduced population of a pure line does not seem probable, or at all possible under the conditions of these experiments. All the basal populations introduced came from cultures in which the reproductive cycle was known to be homozygous with respect to the rhythm of the two generations, and since any heterozygousness in this rhythm is at once manifested and very obvious in the cultures, and was also repeatedly tested for in the stock cultures, and none found, the evidence is against the assumption. Further all the introduced stocks were taken from the original stocks on emergence from hibernation, after all the elimination incidental to hibernation had taken place. Were there lines in the original materials with the conditions that later developed in the Tucson cultures, which were isolated by the selective action of the Tucson experiments, and could not later survive the Chicago conditions when returned thereto; why, if present in the original stocks, were they not also eliminated by the conditions of the winter hibernation in the parental habitat?

Further in the elimination incident to hibernation, that found in preparation for hibernation remains about a constant percentage (about 11) throughout the series at Chicago and Tucson, the difference in the two is in the elimination that takes place during hibernation. The introduced populations at Tucson were all of individuals that had survived the Chicago winter's hibernation, whereas the materials returned to Tucson for testing did not survive the Chicago winter, as shown in the records of the tests.

There is, therefore, no evidence nor reason to interpret the results of the experiments as being due to the isolation at Tucson of pure lines from the introduced population, and every reason, as shown above, why this interpretation is highly improbable. The pure line concept is so facile and insidious in its possibilities for interpretation, that the result of the series of experiments are easily interpreted to conform therewith.

Since 1910 I have tried to develop pure lines from the materials at Chicago with respect to this water retention, and have not up to the present the least evidence that such exist, nor has the slightest success attended my efforts along that line. I cannot go beyond the facts of experience and must, therefore, conclude that upon the basis of the evidence available, pure lines of the characters developed in the Tucson experiments do not exist in the parental materials, and that the results are not due to the sieve-like action of the Tucson environment upon the introduced population, and I believe that this conclusion is entirely justified upon the basis of facts at present available.

It may be assumed that the condition developed was in correlation with some other characteristic operative at breeding, and that introduction eliminated those not possessed of this trait, but in these introductions, as in the T 99 series, from actual observation the entire introduced population took part in the initial breeding, and nowhere have I found the least indication that there has been any such relation, although I have been upon the lookout for them.

That the results are the product of the selection of favorable or adapted variations surviving from among multifarious variations, regardless of size, a product of natural selection, is without any confirmation. The chief change takes place between introduction and the first period of hibernation in the elimination during hibernation, and there is therefore no opportunity for the operation of such selective action, even though it is present in the following two or three hibernations. I have tried to devise tests of this point, thus far without any success, and rather extensive breeding of pedigree pairs from these cultures have thus far given only negative results in this line. While I admit its possibility and its value as a basis for argument or interpretation, I am still without any evidences at all that can be used either way concerning this possibility, and am of the opinion that it is not, in view of the findings, a plausible interpretation of the results.

Concerning the possibilities of the reactivation of latent agents, under the conditions of experiment, a plausible interpretation could be made, if the initial assumption is made that such latent agents exist, an assumption that might receive some support from the probable phylogenetic history of these animals. Were any such condition latent in these beetles, it would seem that some indication would have been found of it in the rather extensive experiments that have been in progress in the last ten years, especially those that have to do with the reproductive cycle and hibernation; but as far as I am aware, there has been no indication thereof, and finding of it would have been of considerable value in many of my experiments, and after all the finding of the latency would not in this instance be of much service in the interpretation of the results.

It seems to me that the most plausible interpretation of the results of these experiments is that we are dealing with a case of the response of the entire introduced population, of uniform composition and behavior, in a determinate variation in Darwin's sense. A population reaction to the conditions of a new environment in a characteristic that is capable of existence, and does exist in species now living in alternative conditions depending upon the environic constellation encountered. That the alteration is directly adaptive in its manifestation implies neither purpose nor use, only that in the relation of water in the tissues, mechanistic considerations show at once that only two possible conditions can exist; to hold the water or to let go of it, and there are no other possibilities. In other words, the alteration is adaptive and directive in its response, because from the physical nature of the materials no other relations are physically possible.

A crucial question is, why has the reaction not been as easily reversible on return to Chicago conditions? In this lies interesting possibilities for investigations now in progress. Up to the present the reversibility of this action has not certainly been obtained, although I have no doubt but that if I can discover the proper relations between the internal mechansim of these beetles and their medium that the reverse alteration of the Tucson condition to that at Chicago will be found.

Whether the reversal ancestralward, or what may be assumed to be an ancestralward direction, is easier than progressively, is for the present is a matter of opinion, and I am unable to discover any physico-chemical reason why it should be so, but we know many reversible actions that go easily through one series of events in one direction, and with more difficulty in the reverse and through another series of operations. In these purely physical and chemical reactions, ancestral influence is not at all involved, and I see no reason to assume the influence of ancestry and phylogenetic relationship in living reactions of the same general physical type.

EVOLUTIONARY SIGNIFICANCE.

In the evolution of populations, in their habitat adjustments as in their distributional phenomena, reactions, accommodations of this sort may well have played a by no means minor rôle, and the value of adjustments of the kind found in these introduced populations at Tucson in these operations in nature are at once too obvious to need mention. Physiological adaptations of many sorts may well have their origin and owe their diversification to operations of precisely this general type, the fact of their heritability or behavior Mendelianwise having no significance or indication of the method of origin. In fact this and other experiences with these animals through many years strongly inclines me to the conclusion, that Mendelian alternativeness is no certain criterion that they arose discontinuously. Osborn has presented from the findings of the fossil record, certain facts concerning the phylogeny of characters in mammals that now behave Mendelianwise in crosses, but, which arose, as far as the data of paleontology can decide, by gradual and continuous modification, and not by mutative jumps, and further in that the Mendelian reactions concern only extremes or alternatives in the same quality of the organism, it may result that the entire Mendelian phenomena are but the expressions of characters whose manifestation is capable of easy and reversible manifestation.

The fact that this alteration is inheritable at all, may to some, seem incomprehensible, but it never segregates, its behavior in heredity is always that of a mono-hybrid, and in all experiences it behaves as a property characteristic of the whole, and possibly involves, therefore, an alteration not of determiners in the usual sense, but an alteration of the colloidal matrix of the tissues, soma and germ, which we must regard not as different kinds of substance, but as different expressions of one and the same specific kind of living material.

From this series of experiments, operations that are important in population evolution, especially of introduced populations, are certainly discovered, and has served in my hands for an entering wedge into the experimental investigation of questions concerning the evolution of populations, an aspect of evolution of which there is extremely little experimental analysis or investigation. In nature and the diverse products of the evolution phenomena in nature, our classification, schemes of phylogeny, our experiences are with populations of diverse things, in associated relations, both living and non-living, and more intimate knowledge of, and a broader understanding of these population problems seems most necessary for further progress in evolution investigations.

As an example of the manner, and the change necessary, and its production in the alteration of an organism originally characteristic of a mesophytic habitat into one in all ways adjusted to the rigors of the Arizona deserts, of the nicety of adjustment, its promptness, and direct adaptive nature, these experiments provide illuminating examples and the basis for continued investigation of the same general aspect of the evolution problems.

Desert Botanical Laboratory,
Carnegie Institution,
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April 17, 1917.

ON THE CAPACITY FOR FERTILIZATION AFTER THE INITIATION OF DEVELOPMENT.[1]

CARL R. MOORE.

I. An Attempt to Fertilize Sea-urchin Eggs Subsequent to Hypertonic Parthenogenesis.

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I. Introduction.

The purpose of the present paper is to present a part of the results of a study of the capacity for fertilization of eggs of the sea-urchin (*Arbacia punctulata*) after the initiation of development by artificial agents. A previous paper[2] by the writer has dealt with some of the phenomena encountered in an attempt to fertilize the eggs of *Arbacia* after a treatment with certain artificial stimuli, *e. g.*, butyric acid and heat. The ideas involved resolve themselves into the question—can eggs that have been activated by artificial parthenogenetic agents, be fertilized by spermatozoa of the same species; or does activation by hypertonic sea-water preclude fertilization as in the case of sperm activation?

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[1] From the Marine Biological Laboratory, Wood's Hole, Mass., and the Hull Zoölogical Laboratory, University of Chicago.

[2] Moore, '16.

The egg of the sea-urchin cannot be fertilized before maturation has taken place, even though the spermatozoön may enter the egg. Penetration is not fertilization. However, if a spermatozoön enters immediately after maturation, normal changes characteristic of fertilization are produced. But shortly after the act of fertilization the egg returns again to a condition in which fertilization is impossible. E. B. Wilson[1] has demonstrated for *Cerebratulus* that if pieces of fertilized eggs are dropped into sea-water containing active spermatozoa there is no reaction between the pieces of eggs and sperm; however, with pieces of unfertilized eggs there is a reaction. Delage, too, has noted that starfish eggs which had been exposed to artificial parthenogenetic agents could not be fertilized.[2]

Maturation, then, leads to changes within the egg that make possible initiation of development by either a spermatozoön or an artificial stimulus. And if this initiation of development is brought about, carrying with it of course a change of the egg system, fertilization is thereby rendered impossible.

It would seem strange not to suppose that an egg passes through essentially similar physiological changes during the development from one cell to a swimming pluteus, whether activation has been produced artificially or by a spermatozoön. If activation by a spermatozoön produces a physiological state of reactivity that leads to development, and, if activation by artificial agents also produces physiological changes that lead to the same kind of development, should we not suppose that the egg—the only common factor—would pass through essentially similar conditions in the two cases? One would judge from the papers of Loeb, that this supposition is erroneous, that sperm will react with artificially activated eggs in an entirely normal manner, producing normal swimming larvæ, while it is positively known that such is not the case when activation has been previously affected by means of a spermatozoön.

The writer has presented proof that eggs of the sea-urchin activated by butyric acid, and resulting in the production of normal membranes, will not react with sperm nor become fertilized, as Loeb has believed,[1] but that they are entirely unresponsive to sperm, even though spermatozoa pass into the egg substance. The present paper deals with an attempted analysis of the conditions that are produced, after activation by hypertonic sea-water, with reference to the capacity of the egg for fertilization.

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[1] Wilson, '03.

[2] Delage, '01.

Loeb[2] determined that an exposure to hypertonic sea-water (50 c.c. sea-water + 8 c.c. $2\frac{1}{2}$ m. NaCl) for a certain period of time would cause sea-urchin eggs, after their return to normal sea-water, to segment in quite a normal manner: they would also undergo further developmental changes and become essentially normal plutei. But certain eggs from the culture will not have had a sufficiently optimum exposure to bring out their full developmental tendencies and will as a result of this remain quiescent after dividing into two, four or eight cells Can these eggs activated by hypertonic sea-water yet be fertilized by a spermatozoön? Loeb says they may be and uses the assertion as a proof that the egg has not been changed in its power of normal physiological reactivity; and that it may be fertilized if only a spermatozoön reaches the cortex of the egg. His hypothetical "lysin-like" substance contributed by the spermatozoön can still assert itself by producing cytolysis and his "corrective agent" of the spermatozoön may also act to check the imposed cytolytic condition and allow the egg to go on to development.

If the conditions are as Loeb believes, we will naturally have to assume that activation of the egg—the initiation of development—is accompanied in the two instances: (1) in sperm activation and (2) in activation by artificial parthenogenesis, by a different series of reactions that lead to the same end, namely the production of swimming larvæ. But if this is so we will have to assume a lack of harmony in the development of an egg when activated by different agents. In the vast number of cases in which development may be artificially initiated there is no common factor but the egg itself. Development is a series of reactions on the part of the egg whether initiation is given by a spermatozoön or by an artificial agent and, unless we assume such a relatively restricted set of reactions, we have as yet no explanation to offer for parthenogenesis. The principal issue at hand is, can an egg of *Arbacia* that has been once activated by hypertonic sea-water be fertilized or reactivated by a spermatozoön of the same species?

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[1] Loeb, '13.

[2] "Art. Par. and Fert.," pp. 237–238.

The results obtained from this study show conclusively that a treatment of the sea-urchin egg with hypertonic sea-water for an optimum length of time—*e. g.*, that in which one obtains the highest percentage of development, both in regard to cleavage and to larval production—leads to changes in the physiological state of the egg that result in the loss of its capacity for fertilization. Spermatozoa readily enter the eggs but they are unable to cause a reactivation of the egg; they appear only as inert bodies within the cytoplasm. Not only does the spermatozoön fail to produce activation changes but also the initial changes, produced by the hypertonic treatment, have created an environment in which a spermatozoön very quickly goes to pieces.

If an egg has not received the optimum exposure for activation, from the hypertonic treatment, a spermatozoön is capable to some extent of provoking activation changes, and a very striking gradient of activity on the part of the egg can be demonstrated from the normal behavior in fertilization to a complete state of responsive inactivity. If the previous treatment has led to segmentation, spermatozoa readily enter the blastomeres, but are entirely incapable of reactivating them.

And finally, the capacity for fertilization has been found to correspond with the presence or absence of the egg secretion, fertilizin. If the egg possesses any capacity for fertilization after the hypertonic treatment, fertilizin has been found to be present; when the capacity for fertilization is negative, no fertilizin has ever been detected by its sperm agglutinating properties.

II. Material and Methods.

The material used in this investigation has been confined to the Atlantic sea-urchin (*Arbacia punctulata*) found in the Woods Hole region. Fresh material was received daily from the laboratory live-car and was kept in running sea-water in the laboratory until used. Eggs and sperm were secured in the usual way—by cutting away the leathery oral disk and collecting the eggs and sperm in separate syracuse dishes as it is shed by the animal. Eggs were always washed in sea-water from two to four times before being used in an experiment, as this insures a more consistent lot of fertilizations than with unwashed eggs.

The method for testing the capacity for fertilization of eggs that have been activated by a hypertonic solution is the same as that employed by Loeb[1] in his studies of this nature. Eggs after washing were transferred to the hypertonic sea-water and allowed to remain for definite periods of time: at the termination of the exposure they were removed with a pipette to two separate dishes of sea-water (150 c.c.–200 c.c.) A and B. Lot A was allowed to stand at room temperature without further treatment, while to lot B was added a quantity of a fresh sperm suspension. For each experiment both a fertilized and an unfertilized lot were set aside as controls.

III. Observations of Living Material.

Since there is a great difference in the effects of different concentrations of, and variable time exposures to, hypertonic sea-water the experiments with the sea-urchin egg have included quite a range of concentrations and periods of exposure, but to simplify the data, only results from two concentrations of the hypertonic solution will be reported at this time. The two concentrations may then be known as the weaker solution (50 c.c. sea-water + 8 c.c. $2\frac{1}{2}$ m. NaCl) and the stronger solution (50 c.c. sea-water + 16 c.c. $2\frac{1}{2}$ m. NaCl).

These two concentrations of hypertonic sea-water were employed because of the very different results obtained with them. An exposure to the weaker solution results in the segmentation of a relatively few eggs (see Table I. and Fig. 1, *a*) but usually a very small percentage of the eggs will segment once or twice and cease their development, while a similar exposure to the stronger solution gives a much higher percentage of cleavages and a much greater number of swimming larvæ. An exposure to the latter solution is much more nearly an optimum treatment for activation than is the weaker concentration.

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[1] "Art. Par. and Fert.," pp. 235–236.

1. *Fertilization after Exposure to Hypertonic Sea-water.*

The fundamental interest of the writer has been directed towards the capacity of an egg, once activated by an artificial stimulus, for fertilization. If an egg is fertilized normally, the spermatozoön supplies the optimum stimulus for the initiation of changes, latent within it, that lead to normal development. But since these same changes may be initiated (it seems that we must regard it so) by artificial agents, is there yet the possibility of an observable stimulus from a spermatozoön if applied subsequent to or during these changes? Can fertilization be superimposed upon hypertonic parthenogenesis?

Eggs were exposed to the action of hypertonic sea-water as given above, returned to normal sea-water and divided into two lots, one of which remained standing at room temperature, while to the other was added a fresh sperm suspension. Let it again be brought to mind that exceedingly variable results are obtained from the same type of treatment of two different lots of eggs and since the condition of the eggs before sperm are added is the all-important factor for subsequent fertilization one must be very careful to know as thoroughly as possible the condition of the eggs in question. In one experiment one may obtain only 3 per cent. of cleavages after a given hypertonic treatment and at another time, under as nearly as possible identical conditions, 25 per cent. to 35 per cent. cleavages. The following experiment will indicate the method of procedure and the results in general that have been obtained.

From the results of this experiment one can readily see the different capacities for fertilization possessed by eggs exposed to the weaker solution (lot A) and those activated by the stronger one (lot B). This relation can be seen at a glance from Fig. 1, the curves of which were plotted from this experiment.

The lot of eggs returned to normal sea-water and inseminated, after an exposure to the weaker solution A, for a period of ten to twenty minutes, appear very little, if any, different from the control lot of normally inseminated eggs, either as regards

Experiment:

4:30 P.M. Eggs collected and washed. Aug. 26, 1916.
5:00 P.M. Divided into two lots *A* and *B*.
A, placed in (50 c.c. sea-water + 8 c.c. $2\frac{1}{2}$ m. NaCl).
B, placed in (50 c.c. sea-water + 16 c.c. $2\frac{1}{2}$ m. NaCl).

Eggs were returned to sea-water from the two solutions by means of a pipette and divided into two lots one of which remained without further treatment (control): to the other was added immediately a fresh sperm suspension.

Table I.

Lot A.

No.	Returned to Sea-water.	Hypertonic Solution Alone.		Hypertonic Solution Plus Sperm.	
		Percentages of		Percentages of	
		Cleavages.	Swimming Larvæ.	Cleavages.	Swimming Larvæ.
1	5:10 P.M.	0	0	99	90
2	5:20 “	0	0	95	85
3	5:35 “	1	0	83	70
4	5:50 “	2	0	70	55
5	6:00 “	3	1	60	35
6	6:20 “	2	0	28	20
7	6:35 “	2	2	33	20
8	6:50 “	4	0	30	15
9	7:05 “	5	1	28	5
10	7:20 “	4	1	20	15
11	7:35 “	6	0	35	10
12	7:50 “	8	0	35	5

Lot B.

1	5:05 P.M.	1	0	22	25
2	5:10 “	0	0	18	18
3	5:20 “	10	7	12	20
4	5:30 “	22	20	20	28
5	5:40 ‘	18	7	18	5
6	5:50 “	14	5	9	2
7	6:00 “	12	1	10	1
8	6:20 “	5	0	1	0
9	6:35 “	6	0	6	0
10	6:50 “	4	0	1	0
11	7:05 “	2	0	2	0

the number of cleavages or the number of swimming larvæ. But since an exposure of this intensity is practically ineffective in producing initiation of development, is it not to be supposed that the eggs can yet be fertilized by sperm? They are, so far as we may judge, entirely normal or essentially so. However, as the length of exposure to the hypertonic solution is prolonged the capacity for fertilization drops off until after an exposure of two hours' duration scarcely any larvæ appear as a result

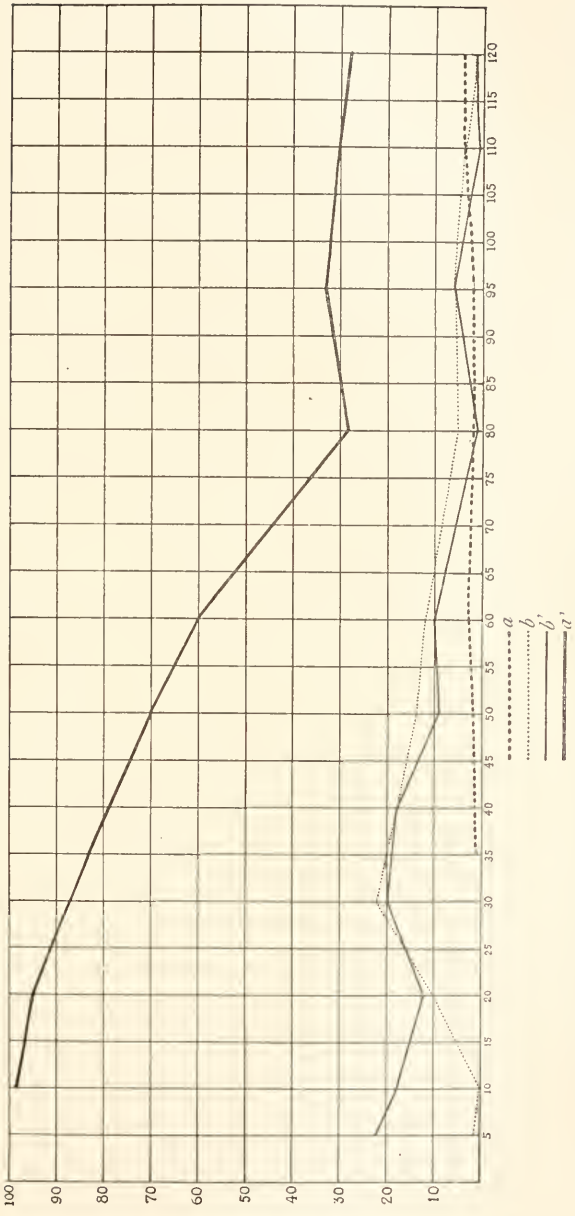

FIG. 1. Curves showing the initiation of development of *Arbacia* eggs as measured by cleavages, after an exposure to hypertonic sea-water; and the relative capacity for fertilization after an exposure to the different concentrations. *a*, weaker hypertonic solution alone; *a′*, weaker hypertonic solution followed by insemination; *b*, stronger hypertonic solution followed by insemination; *b′*, stronger hypertonic solution alone. The ordinates represent percentages of cleavage; and the abscissæ, time of exposure in minutes.

of insemination.[1] This is undoubtedly due to the condition of the egg, produced as a result of the hypertonic exposure· its capacity for fertilization is being gradually decreased.

We may correlate, it seems, this gradually progressing unfertilizable condition of the egg that appears as the eggs are allowed to remain in the solution, and the escape of substances from the eggs which also increases as the eggs are allowed to remain for longer times in the solution. One can readily notice an almost entire absence of pigment from many of these eggs while others will show a decided loss of it. Probably also many other substances escape at the same time from the egg, some of which may be absolutely necessary for certain chemical combinations taking place during fertilization and development.

The type of swimming larvæ obtained in such a series of experiments shows also a very beautiful gradient in normality as well as in number. Most of those obtained as a result of fertilization, after a short hypertonic treatment, appear entirely normal, but the percentage of abnormalities increases as the length of exposure to the hypertonic solution before fertilization was lengthened: after an exposure of 1½ hours to 2 hours practically no normal larvæ are obtained and the mortality is very high.

For the higher concentration the curve (*b* and b^1, Fig. 1) is decidedly different from (*a* and a^1), the weaker one, but yet it is quite characteristic for exposures to such a concentration. The greatest percentage of cleavages from the hypertonic solution alone are obtained in lots, the exposure of which had been thirty minutes or forty minutes in duration while often a small amount of segmentation is induced by an exposure of 1½ hours. But distinctly different from the curve of cleavages when the lower concentration was used is the fact that the curve of fertilization (?) follows more nearly that of the hypertonic treatment alone. This is especially true when the percentage of swimming larvæ is considered. In dishes of inseminated eggs, previously exposed to the stronger hypertonic solution for 10,

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[1] The number of cleavages after a two-hour exposure in this experiment is exceptionally high as compared with scores of other experiments for the same length of time. Usually such an exposure is followed by not more than 3 per cent. of swimming larvæ but many times swimming forms do not appear at all.

20, 30 or 40 minutes, quite a goodly percentage of larval forms are to be seen, but compared with the curve of larval production of the hypertonic solution alone it has been increased very little or not at all. We cannot, therefore, refer the swimming larvæ appearing in these inseminated cultures to fertilized eggs but they can be regarded only as larvæ that have developed from the action of the hypertonic solution upon the eggs. Insemination did not increase the number of cleavages or swimming larvæ over that obtained after hypertonic activation alone.

We can now consider more in detail the results of the experiment under consideration and let it serve to illustrate the results of most of the experiments.

In an exposure to the weaker concentration of hypertonic sea-water very few eggs give evidence of a cortical change that in any way resembles the fertilization membrane produced around the egg when normally fertilized, and in keeping with this, very few of the eggs segment. It is difficult to say whether or not every egg that cleaved had previously undergone a cortical change. It is difficult even to say if an egg has cleaved or not, for the cleavage patterns have been so variously modified, are so irregular and these are scattered promiscuously among so many eggs that are undergoing degeneration that it is very difficult to determine whether an egg has segmented or is slowly falling to pieces. Many times the cytoplasm of the egg pulls apart, leaving a wedge-shaped opening that finally separates the egg into two approximately equal halves with the nucleus contained entirely within one half; and yet at other times nuclear division may occur without cytoplasmic division. But that there is not a membrane produced that will hold the blastomeres together as in normal fertilization is very evident from the fact that even the first two cells may fall apart and become entirely separate from each other or be held together only within the clear transparent jelly layer of the egg—the chorion layer. But a small percentage of the eggs do produce slight membrane changes: the membrane appears around the egg and can be easily seen with the higher powers of the microscope. They are slightly thickened, clear jelly-like membranes adhering very close to the egg and are not at all to be confused with a normal membrane. It is therefore possible to assume that all eggs that have segmented have been preceded by cortical changes of some intensity at least.

As one may see from Table I., the largest percentage of cleavages obtained from an exposure to the weaker hypertonic solution (Lot A) was 8 per cent. and that the greatest number of swimming larvæ was 2 per cent., therefore many of the eggs had segmented that did not continue their development to the swimming stage. The optimum effect of the solution for initiation of development was not imparted to the eggs and development ceased after a few cleavages. In the great majority of eggs, however, no evidence of initiatory changes are discernible and one would naturally suspect that they were yet capable of fertilization, and this, as the experiment shows, is partially true. The shorter exposure eggs when inseminated immediately after return to sea-water, react quite normally with sperm, producing a fertilization membrane, normal cleavage and a high percentage of swimming larvæ. But as the exposures are lengthened there is a gradual decline of the normal conditions until an exposure to hypertonic solution, sufficiently prolonged, will entirely prohibit fertilization of the eggs.[1] After an exposure, lasting 25 to 30 minutes, to this concentration, the eggs begin to show a tendency for loss of pigment: they are lighter in color and a few are so affected as to be reduced to mere shadows of their former condition, but this is much more true of the longer exposures.

The character of cleavage and of larval development also declines from the normal as the curve of fertilization approaches zero. In the experiment cited, dishes A 1 and A 2 gave practically normal cleavage and 85 per cent. to 95 per cent. of swimming forms that appeared practically normal. Beginning with dish A 3 however and extending throughout the series of exposures, cleavage became less and less normal; the cleavage planes had grown more indistinct and in many eggs division of the nucleus had gone on while the cytoplasm remained unsegmented. Some of the dishes presented such an indistinct lot of cleavages that it was almost impossible to judge whether or not an egg had cleaved but the cleavage count is as nearly correct as very careful examination could make it. The larvæ appearing in dish A 1, after insemination, were a good lot: approximately 70 per cent. swam at the top of the water and more than 50 per cent. are entirely normal. Compared with the swimming forms of A 5, obtained after fertilization, there is a great difference: only a small percentage of the eggs subjected to the hypertonic solution for one hour (A 5) and inseminated immediately, gave rise to swimming larvæ; and of these only a few could be seen swimming at the top of the water. Almost any form of moving masses of protoplasm may be found among the forms of this dish from a very few apparently normal forms to masses that gave only the least bit of quivering movements; blunt masses, angular forms possessing no semblance of morphological similarity to a normal form, dumb-bell shapes one half of which swims and pulls the other lifeless half around with it, bits of spinning protoplasm that appear somewhat as half, quarter or eighth blastulæ are to be found abundantly. As the length of exposure to hypertonic solution is increased before insemination one encounters a more and more abnormal lot of larvæ; there is not only an increase in the degree of abnormality but the percentage of swimmers is gradually reduced until dish A 11 (inseminated) contained only 10 per cent. of eggs that gave the least signs of life, and of these not any swam at the surface of the water; A 12 (inseminated) revealed not a single form that approached the normal.

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[1] Lack of fertilization after exposure to solutions of various salts, has been pointed out by R. S. Lillie, '11.

It is well to point out again the inability of one to obtain an absolutely correct larval count, for among the eggs that have not been fertilized are many that have undergone cytolysis; the count is only relatively correct but it will be sufficient to illustrate the decrease of the capacity for fertilization of eggs that have been exposed to the activating influence of the weaker solution of hypertonic sea-water.

When the more concentrated solution is used as an activator and is followed by insemination, a different condition is encountered. After the hypertonic treatment alone, of say thirty minutes, one obtains a much larger percentage of cleavage and a decidedly higher percentage of swimming larvæ than is obtained after the same exposure to the weaker solution. But a comparatively short exposure (5 to 10 min.) to the stronger concentration results in the production of quite a considerable number of membranes. The membranes too are decidedly more pronounced than those produced after exposure to the weaker solution but are not yet like the fertilization membrane produced in normal fertilization, even though the physiological processes responsible for their production may be analogous· except perhaps in intensity. Cleavage, after this exposure, although departing very greatly from the normal, is not characterized, as are many of them after the weaker solution, by the separation of the blastomeres; the cells remain in close proximity to each other—a fact that may possibly be associated with the more rigid membrane that is produced around the egg—and a larger percentage of them reach the swimming stage than of those from the other solution. But here also as the length of exposure is increased and the curve of cleavage falls, the same decline in degree of normality and of percentage of swimming larvæ is apparent.

In keeping with the more pronounced effect of the stronger solution in the production of membranes is the fact that the eggs suffer a greater increase in permeability. Loss of pigment is more pronounced than in the case of the weaker solution as one would expect. A small percentage of swimming larvæ obtained after a relatively short exposure to the stronger solution are somewhat normal, but the lots grade so gradually into abnormality and immobility that many can be seen to possess but very little power of movement. Many half, quarter or eighth sized larvæ are found here, as well as following an exposure to the weaker concentration.

There is revealed here a general shake-up of development that is quite characteristic, not merely for the hypertonic treatment, but for development after treatment with any agent that tends to destroy the delicate physico-chemical balance of an egg.

This experiment which may be considered a typical one, offers no indication that the addition of a sperm suspension to eggs that have been activated by hypertonic sea-water are yet capable of being fertilized, but on the contrary many eggs that have failed to respond outwardly to the activating influences, by segmenting, are found to be incapable of fertilization. The reaction differences of the eggs or their different physiological states assert themselves by the variety of reactions given by the egg after insemination; one egg will segment and produce a swimming larva; another will divide a few times but finally disintegrate; yet still another will remain to all appearances in a normal condition, being normal in size and color, will be surrounded with spermatozoön and yet does not divide; while finally one will retain its full rounded appearance but give up practically all its pigment content to the surrounding water, but does not segment. If the eggs are given the optimum exposure for cleavages,[1] insemination does not increase either the percentage of swimming larvæ or of cleavages.

Professor Loeb, however, makes the claim that sea-urchin eggs may undergo activation, from an exposure to hypertonic sea-water, to such an extent that they segment once or twice, as the case may be, but are nevertheless still capable of fertilization. Quoting from Loeb:[2]

"When we put the unfertilized eggs of *Strongylocentrotus purpuratus* directly into hypertonic sea-water . . . and if we put them back at different intervals into normal sea-water, we find that if eggs have been exposed a sufficiently long time (two hours or more) to the hypertonic sea-water a number will begin to segment. These eggs will often go into the two- or four-cell stage, or sometimes to the eight- or sixteen-cell stage, and then stop developing . . . such eggs remain after this perfectly normal and they have the appearance of small unfertilized eggs. If we wait for sometime, say twenty-four hours, to make sure that they neither develop nor disintegrate, and add sperm, each one of these blastomeres forms a tightly fitting membrane. They begin to develop in a perfectly normal way and into normal larvæ."

Whether or not the fundamental principles of development in the egg of *Arbacia* differ from those of *S. purpuratus*, the results obtained by the writer with the eggs of *Arbacia* do not agree with the observations of Professor Loeb on the eggs of the California sea-urchin.

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[1] In this particular experiment an exposure to the stronger solution for 30 minutes.

[2] " Art. Par. and Fert., p. 237.

The eggs of *Arbacia* will, as does the above cited instance of behavior of the *Stronglyocentrotus* egg, segment into two or four cells and go no farther in development, but remain for several hours apparently normal half or quarter eggs. This condition is perhaps best obtained in *Arbacia* following a hypertonic treatment of thirty to forty minutes, with the weaker solution. If such a lot of eggs are returned to normal sea-water and allowed to remain at room temperature (21°–23° C.) for twenty-four hours, many of them will appear quite normal, while all gradations of loss of pigment can be found. There are present also many small eggs of half or quarter normal size but the majority of these are not normal; most of them are entirely or partially devoid of pigment; some few of them however do appear normal in color. If sperm are added to such dishes and the effects of the insemination carefully noted, a few very striking facts are to be observed:

(1) The smaller eggs as well as the eggs that have not segmented are surrounded by actively swimming spermatozoa but they do not produce a fertilization membrane;[1] (2) many of the unsegmented eggs will segment, but the blastomeres are not held together. Since a good membrane is not produced, the blastomeres may become entirely separated from each other or be retained within the very thin jelly layer of the egg and not be in contact with each other as they are normally. These blastomeres may again divide giving rise, each, to two daughter blastomeres that may again become separate, making four individual blastomeres from the same egg or four quarter-eggs; and these may go on dividing until a great mass of cells are present but not held together. Some few of them (for instance all those derived from one of the first two daughter cells) may remain in loose approximation and perhaps give rise to a very

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[1] In many cases eggs that have been exposed to the weaker concentration for a short time only, will produce a quite typical membrane after insemination, but those exposed for one hour to this solution, and allowed to remain standing for twenty-four hours, are almost entirely devoid of any semblance of membrane production after insemination.

abnormal and much smaller swimming larva than the normal. On the other hand, the writer has watched for long periods of time the half- or quarter-eggs found in the dish at the time of insemination but has not found for certain a single one of these that produced a membrane or has segmented as a result of fertilization. But if one should only casually observe the eggs following such a treatment there is no way to distinguish between the separated blastomeres of an egg that has been fertilized and has segmented into two blastomeres that have become separate, and one of the half- or quarter-eggs that was present at the time of insemination. Of importance also is the fact that the *character of a swimming larva of reduced size found in these inseminated cultures is no criterion that would indicate from what kind of an egg it was derived.* As before stated, swimming larvæ of all sizes and of most any external shape assumed by protoplasm can be found in dishes, the eggs of which have been exposed to the hypertonic treatment alone. How then may we conclude that a half- or quarter-sized larva obtained in such a mixed culture after insemination, was derived from a half or quarter-sized egg by fertilization? The only possible way in which the writer could know the exact conditions of the egg from which the swimming larvæ came was by the very laborious method of isolation of individual eggs, and this method was adopted as a last resort.

Four or five hours after an exposure to the weaker concentration of hypertonic sea-water one often finds that 2 per cent. or 3 per cent. of the eggs have segmented once, producing two equal blastomeres that are separated from each other but remain enclosed by the thin transparent jelly layer of the egg. By means of a very fine capillary pipette connected with a flexible rubber tube held in the mouth, these two separate cells, or half-eggs, were isolated from the cultures while in focus under a binocular microscope and collected into a small dish. Thus it was definitely known that only blastomeres of one kind were present. These isolated half-eggs could then be observed and be inseminated in a pure culture, and hundreds of them were isolated and studied in this manner, enabling the writer to determine the following points:

(*a*) Not a single blastomere of the first cleavage of an egg (a half-egg) isolated and left standing in normal sea-water at room temperature, appeared entirely normal at the end of twenty-four hours.

(*b*) A few of these blastomeres, without further treatment, segmented again and some many times. This cleavage was often delayed some hours after treatment, indicating that developmental processes were going on very slowly.

(*c*) Not a single normal larva was obtained out of hundreds of these isolated blastomeres inseminated seven hours or longer after the treatment.

(*d*) These inseminated, apparently normal blastomeres did not form a fertilization membrane.

(*e*) Out of the hundreds of half-eggs that have been inseminated less than 10 per cent. have ever segmented. It is just possible that some of these cleavages have been due to activation by spermatozoa, but the eggs had been so weakened that cleavage was abnormal and never reached the swimming stage. *But beyond doubt most of these cleavages were due to the slow development going on within the egg similar to* (*b*) *above.*

With these facts in mind is it not possible to account for the results of Loeb without considering that his half- or quarter-larvæ came from the fertilization of the separated blastomeres of the 2- and 4-celled stage? As pointed out above, eggs that have remained standing for 24 hours after hypertonic exposure do not produce membranes after insemination, that are rigid enough to hold the blastomeres together; and in these experiments, as well as in Loeb's, many dwarf and badly deformed larvæ can be seen in the culture but they do not come from the fertilization of a half- or quarter-egg, but rather from an egg that was fertilized and later lost cells. Numbers of swimming forms can be seen trailing a comparatively huge mass of cells after it, that have been lost from the egg itself and consequently has resulted in a reduction in size. Even in the cultures producing swimming larvæ from the effects of the hypertonic solution alone, one encounters these half-, quarter-sized, and even smaller swimming masses representing parts of eggs, but these too are reduced in size from loss of cells during the division of a whole egg and possibly also from an egg, one half of which may have become separated after the first cleavage.

The fact that among these 24-hour cultures of eggs, insemination is not usually followed by a perceptible membrane, may lead one to consider the second division of an egg as a division of two half-eggs that have segmented since insemination but not as a result of it. If these first two blastomeres from the cleavage of an egg are separated and each divides again, how is one to judge whether this is the real condition or that perhaps they may represent the fertilization of a half-egg? Decidedly not by periodical examination, but only by continual observation, if in a mixed culture, or by isolation; and as has just been pointed out, hundreds of isolated blastomeres have been inseminated in pure culture but not a single swimming form has been obtained from these, that have remained at room temperature seven hours between the time of hypertonic treatment and insemination.[1] Whether this explanation will serve to harmonize the differences of opinion or whether there is an individual variation between the fundamental processes of development in the two different species of sea-urchin eggs, the writer is unable to say.

But to obtain a further insight to the observable effects of the addition of sperm to eggs that have been caused to start their developmental processes, eggs from a large number of experiments have been preserved and sectioned and the cytological results are presented briefly in a following section.

2. *Fertilizin and Fertilization.*

In a former paper the writer considered in some detail the relation of the presence of the sperm agglutinating substance, fertilizin, liberated from the normal eggs of arbacia into sea-water in which they have been standing, and the capacity of eggs for fertilization, both normal eggs and eggs that had been treated with various reagents. It is not the purpose of the present paper to take up for discussion the disputed points of the fertilizin theory[1] nor to discuss the way in which fertilizin acts or its rôle in the process of fertilization, but it is perhaps desirable to add another instance of the existing parallelism between the presence of a detectable amount of this agglutinating substance and the capacity for fertilization. Professor Lillie and the writer have previously shown this parallelism to exist in all cases thus far tested: (1) Normal, ripe *Arbacia* eggs liberate this substance into the sea-water as long as they are capable of fertilization; eggs whose germinal vesicles have not broken down do not liberate fertilizin in detectable quantities and they cannot be fertilized. (2) If *Arbacia* eggs have been exposed to the optimum concentration of butyric acid and full membranes were produced, no fertilizin was detectable and the eggs could not be fertilized. (3) If eggs are over-exposed to the same concentration of butyric acid (1 to 3 mins.) the eggs can be, at least, partially fertilized and fertilizin is readily detected. (4) If they are exposed for two hours to this same concentration of butyric acid (50 c.c. sea-water + 2.8 c.c. N/10 butyric acid) they cannot be fertilized and fertilizin is not liberated in detectable quantities. (5) If eggs are exposed to heated sea-water of 35° C. for 10 minutes they could not be fertilized nor could fertilizin be detected; and finally to be added from these experiments. (6) If eggs are exposed to a hypertonic sea-water solution for one to one and one-half hours, sometimes some of the eggs are still fertilizable to a limited extent but always such conditions have been accompanied by a detectable amount of fertilizin; but in conditions where the eggs are not capable of fertilization no fertilizin has ever been detected. Only one experiment will be given in detail to show this parallelism (see Table II.).

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[1] Some time must be allowed before insemination because of the fact that some of these blastomeres continue to divide before insemination; even seven hours does not entirely eliminate this source of confusion, for a blastomere may divide after that length of time. Developmental changes are still going on, but at a very slow rate.

From microscopical examination at 5:00 P.M., August 15, one could see that many eggs of the X lot were apparently normal in appearance; they were well rounded, and seemed to possess practically the normal amount of pigment, but upon insemination they did not produce fertilization membranes and the blastomeres after segmentation were not held together, and not one of them developed to the swimming blastula stage.

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[1] See Lillie, '14, Moore, '16.

Experiment: Aug. 14, 1916.

2:30 P.M. Eggs collected and washed—divided into lot X and lot Y.

3:05 P.M. X put into (50 c.c. sea-water + 8 c.c., $2\frac{1}{2}$ m. NaCl). Y put into (50 c.c. sea-water + 16 c.c., $2\frac{1}{2}$ m. NaCl).

4:25 P.M. Both lots returned to sea-water and lightly shaken to free from jelly, and series of washings begun.

Table II.

Time.	Designation.	Lot X.	Lot Y.
4:50 P.M.	Washing 1	$\frac{10}{1.0}$[1]	$\frac{10}{1.0}$
5:10 "	" 2	"	"
5:50 "	" 3	"	"
6:00 "	Inseminated	Cleavage 10 per cent.	0
Aug. 15			
8:30 A.M.	Washing 4	$\frac{10}{1.0}$	$\frac{10}{1.0}$
9:10 "	" 5	"	"
10:45 "	" 6	"	"
11:10 "	" 7	"	"
12:30 P.M.	" 8	"	"
2:10 "	Tested for fertilizin	8 second reaction	negative
	Inseminated	Cleavage 13 per cent.	0
2:15 "	Washing 9	$\frac{10}{1.0}$	$\frac{10}{1.0}$
3:00 "	" 10	"	"
3:20 "	" 11	"	"
4:00 "	Test for fertilizin	5 second reaction	negative
4:20 "	Washing 12	$\frac{5.0}{1.0}$	$\frac{5.0}{1.0}$
5:00 "	Tested	4 second reaction	negative
	Heavy insemination	Cleavage 20 per cent. (poor)[2]	0
5:20 "	Eggs killed in Boveri's picro-acetic acid		

The results of this experiment have been confirmed by a number of others and the results have always been consistent—if the supernatant fluid possessed enough fertilizin to cause the agglutination of a fresh sperm suspension, always there had been eggs present in the culture that possessed enough latent developmental capacity to react with a spermatozoön and to segment as a result of this union, but if no fertilizin could be detected never have the eggs been found to possess a capacity for fertilization. Some change in the physiological or physico-chemical state of the egg has been brought about as a result of the hypertonic treatment and fertilization is impossible. Of what this change consists we do not know, but it is at least accompanied by an increased permeability of the egg and loss of substances from it. Whether a specific substance necessary for fertilization has diffused from the egg or whether it has undergone new chemical combinations that have rendered it unavailable and whether it may be the substance called fertilizin are questions that cannot now be answered. But it seems significant that here as in all previously modified conditions there has always been a substance escaping from the egg into the surrounding sea-water that could be detected by its sperm agglutinating properties, if the eggs were still capable of fertilization. That this unfertilizable condition is due to a new physiological state of the egg itself and not to a physical condition of the surface of the egg that might prevent sperm entrance, is made certain by observation on preserved material. Sperm do penetrate these eggs and sometimes produce weak changes similar to fertilization or they remain entirely ineffective, depending upon the condition of the egg itself.

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[1] In every case the numerator of the fraction represents the volume of eggs and sea-water while the denominator represents the volume of eggs and sea-water (in c.c.) remaining in the tube after most of the supernatant sea-water had been drawn off—for further details see footnote 1 on page 276.

[2] No larvæ produced from 5 P.M. insemination, in either lot X or lot Y.

3. *Conclusions of Section III.*

The results of this series of experiments seem to indicate that a certain optimum exposure of the eggs of *Arbacia* to hypertonic sea-water initiates changes within the egg that result in development. That all do not segment is because the optimum exposure for one egg is not the optimum for all.[1] Some have been over-exposed, some under-exposed, but if the exposure was the optimum one, swimming larvæ were produced; if less or greater than the optimum perhaps only one or two cleavages resulted and development ceased; if decidedly over-exposed or under-exposed, cleavage does not occur. This quantitative relation is then interesting from the standpoint of fertilization. If the eggs were not perceptibly affected by the exposure they could be fertilized; if the reactions initiated by the exposure were of sufficient intensity to disturb the physico-chemical condition of the egg and cause the loss of substances necessary for fertilization or has caused new chemical combinations to be formed that renders certain substances unavailable, then fertilization is likewise impossible.

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[1] The difference may be due to the factor of aging as Goldfarb ('16) has applied the term.

In order to determine definitely whether or not this unfertilizable character of the egg is due to a real physiological or physico-chemical condition of the egg substances or whether it is due to physical conditions that prevent the extrance of a spermatozoön into an egg, a large number of these from different kinds of experiments were preserved and sectioned. Usually Boveri's picro-acetic acid was used as a killer and sections (4 μ thick) were stained in iron hæmatoxylin.

IV. Observations from Preserved Material.

The cytological data to be presented is not meant to be a critical analysis of chromosome behavior but is given only to acquaint us with a few of the more general facts of the behavior of sperm in relation to these unfertilizable conditions, and it will be confined to observations upon three different classes of experiments: (1) To mass cultures of eggs that have been exposed to the weaker concentration of hypertonic sea-water for different lengths of time, returned to normal sea-water and immediately inseminated; (2) to mass cultures which have been exposed to the stronger concentration of sea-water for different lengths of time, returned to sea-water and inseminated; (3) to pure cultures of eggs that have segmented but once after an exposure to the weaker concentration, and inseminated.

1. *Insemination Following an Exposure to the Weaker Hypertonic Solution.*

Experiment:

2 P.M. Eggs were collected, washed three times.

2:50 P.M. Transferred to hypertonic sea-water—(50 c.c. sea-water + 8 c.c., $2\frac{1}{2}$ m. NaCl).

Eggs returned at intervals to sea-water, divided into lots A and Ax. Lot A no further treatment; control.

Lot Ax, immediately inseminated after return to sea-water. Eggs from both lots killed as given in Table III.

TABLE III.

No.	Lot A.			Lot Ax.			
	Returned to Sea-water.	Per Cent. Cleavages.	Per Cent. Swimming Larvæ.	Per Cent. Cleavages.	Per Cent. Larvæ.	Inseminated.	Killed.
1	3:10 P.M.	2	1	85	75	3:15 P.M.	3:45 P.M.
2	3:30 “	2	0	75	75	3:35 “	4:05 “
3	3:50 “	15	1	45	15	3:55 “	4:25 “
4	4:10 “	25	3	30	8	4:15 “	4:45 “
5	4:30 “	12	0	12	1	4:35 “	5:05 “
6	4:55 “	20	1	12	2	4:55 “	5:25 “

Microscopical Observations on Lot A.

3:30 P.M., A^1 eggs appear normal.
3:55 P.M., A^2 eggs appear normal.
4:15 P.M., A^3 eggs appear normal.
4:40 P.M., A^4 10 per cent. eggs show loss of pigment.
5:10 P.M., A^5 few eggs almost colorless; 30 per cent. appear normal.
5:20 P.M., A^6 some eggs reduced to shadows, many appear quite normal.

Sections of eggs of the AX^1 lot appear not greatly different from lots that have been normally fertilized; very few of them

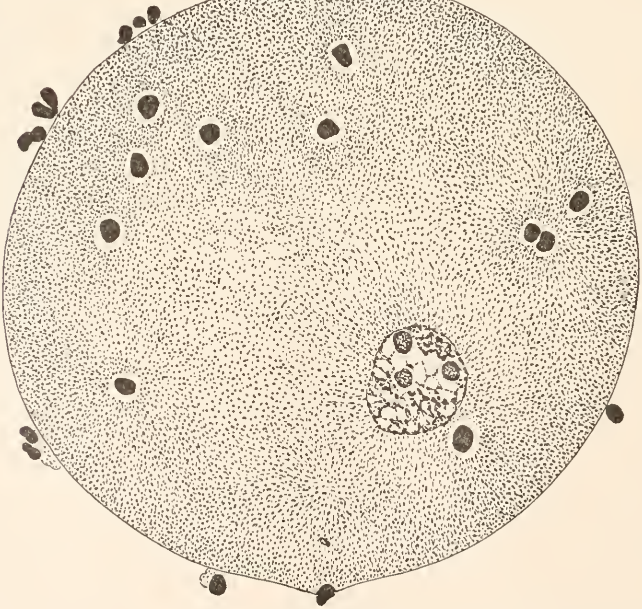

FIG. 2. × 3,000. Section of an egg exposed to the stronger hypertonic solution for 15 minutes and inseminated immediately on return to sea-water. Killed 15 minutes after insemination. The sperm heads are entirely unchanged and have not reacted with the egg.

show more than one sperm nucleus and this has usually undergone the characteristic swelling and vacuolization changes of a male pronucleus and is accompanied by an aster. After a longer exposure to this concentration of hypertonic sea-water the resemblance to sections of normal fertilization gradually disappears and is superseded by a general condition of polyspermy and later by a non-reactive state of the egg substances, with an abundance of spermatozoa, lying within the cytoplasm, entirely unchanged. Of the eggs of AX^3, probably the larger percentage are polyspermic and show a reactive condition of the sperm heads; they appear swollen and slightly vacuolated and are usually accompanied by an aster more or less well formed. Some sections of this lot still appear almost normal in their

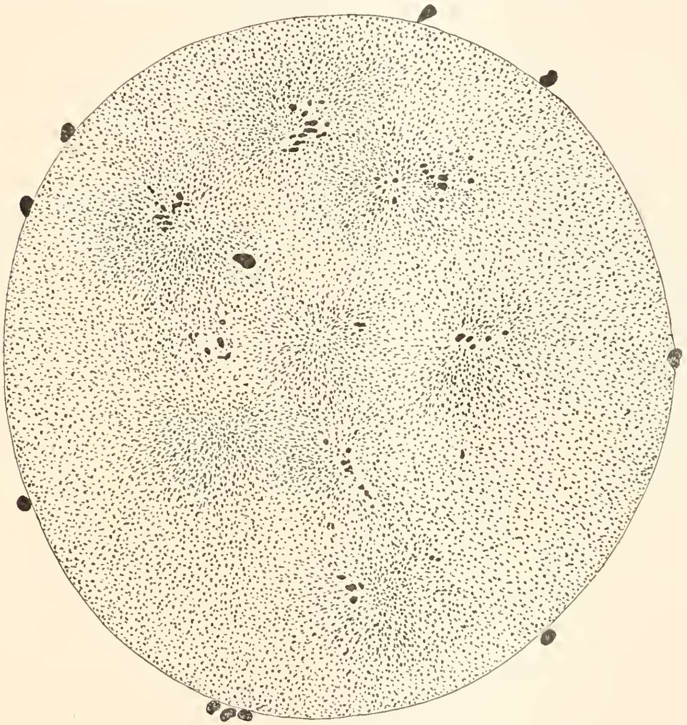

FIG. 3. × 3,000. Section of an egg exposed to the weaker hypertonic solution for two hours and inseminated immediately on return to sea-water. Killed 20 minutes after insemination. All but one of the sperm heads are undergoing fragmentation. The egg nucleus does not appear in this section.

general characteristics. In lot AX^5 however some of the sperm heads are slightly swollen but are not associated with astral radiations and still others are surrounded with weak radiations. Many cases are encountered in which the egg nucleus has broken down into chromosomes, dispersed along the rays of a unipolar spindle, formed by the egg, as a result of the hypertonic treatment; and at the same time several sperm nuclei may lie unchanged within the cytoplasm of the egg.[1] In AX^6 most of the eggs have almost completely lost their capacity to react with a spermatozoön. The majority of the sections show the sperm heads entirely lacking an aster, but many asters produced by the effects of the hypertonic solution are present and are very definite. The reaction between the egg and sperm is largely negative. In numerous sections the physiological changes within the egg cytoplasm have not only prevented changes characteristic of normal fertilization, but they are even of such a character as to cause the disintegration of sperm heads. Fig. 3 is a section of such an egg and shows one sperm head yet unchanged but several others are undergoing dissolution. Other sections show another type of disintegration which is characterized by a swollen vesicle about the sperm head which is breaking apart and stains very faintly with iron-hæmatoxylin.

2. *Insemination Subsequent to an Exposure to the Stronger Solutions.*

The examination of sections of eggs of the following experiment serves to give a general understanding of the incapacity for fertilization, after eggs have been exposed to the stronger solution of hypertonic sea-water.

Experiment:

4:30 P.M. Eggs collected, washed three times.

5:10 P.M. Transferred to hypertonic sea-water—(50 c.c. sea-water + 16 c.c., $2\frac{1}{2}$ m. NaCl).

Eggs transferred at intervals to sea-water and divided into two lots X and Y. Lot X remains standing without further treatment. Lot Y inseminated immediately after return to sea-water. Eggs were removed from each lot at definite times and preserved as given in Table IV.

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[1] See Wilson, '01, and Hindle, '10.

TABLE IV.

No.	Returned to Sea-water	Lot X.		Lot Y.	
		Percentage of		Percentage of	
		Cleavages	Swimming Larvæ.	Cleavages.	Swimming Larvæ.
I	5:15 P.M.	10	0	25	3
II	5:20 "	6	0	12	7
III	5:25 "	5	2	12	3
IV	6:00 "	20	2	22	2

Series Preserved in Picro-acetic Acid.

I x killed 5:30 P.M.	I y^1 killed 5:30 P.M.	I y^2 killed 5:45 P.M.
II x " 5:50 "	II y^1 " 5:35 "	II y^2 " 5:50 "
III x " 5:25 "	III y^1 " 5:40 "	III y^2 " 5:55 "
IV x " 6:15 "	IV y^1 " 6:15 "	IV y^2

The general cytological observations on this preserved series are as follows: In all lots of eggs exposed to the stronger concentration, from five minutes to fifty minutes, and inseminated immediately upon their return to sea-water, sperm have penetrated the eggs freely and lie in any plane of the egg cytoplasm from the periphery to the center. No attempt has been made to determine by actual count, from sections, the relative proportion of eggs containing spermatozoa, but this condition is found under every field of the microscope and many sections of eggs show from two to sometimes twenty sperm heads. By far the larger number of eggs show the presence of sperm heads imbedded within the cytoplasm.

The effects of the egg cytoplasm environment upon the individual spermatozoön varies in the different eggs in which they are found. Many sections prove by their presence that three, four or more spermatozoa have entered the egg and react with it, thus giving the picture of a typical case of polyspermy; the sperm heads may be separated from the egg nucleus and accompanied by a sperm aster, or two or three may have copulated with the egg nucleus. In other sections a dozen spermatozoa may be scattered throughout the cytoplasm, but thirty minutes after insemination they exhibit not the slightest perceptible change in size or shape (see Fig. 2). They appear perfectly solid with no indications of vacuolization; there is no indication of a sperm aster, and in short the sperm heads appear only as inert foreign bodies in the cytoplasm of the egg.[1] Still other sections exhibit a different type of reaction of the sperm to its new environment, that appears significant—the sperm heads may undergo swelling and vacuolization without any indication of the appearance of a sperm aster. For the most part asters arise only in connection with a spermatozoön that shortly appears to be in the swollen, rounded, and slightly vacuolated condition but, as has been mentioned, some possess much more pronounced astral rays than others. This condition of the entire absence of a sperm-aster from the spermatozoön undergoing the more or less characteristic changes of activity, probably to be referred to chemical influences exerted upon it by some substance within the egg, seems to indicate a quantitative reaction of the spermatozoön with the egg, the sperm aster formation being the more sensitive factor. In fact this series of preparations exhibits a gradient of the effects of a spermatozoön, from essentially its normal effect, to a condition in which it provokes not the slightest change; and since a sperm aster is usually associated with a swollen sperm head cannot this be an indication of the suppression of sperm aster formation but a continuation of the more stable changes accompanying sperm activity?

(*a*) *Penetration of the Spermatozoön.*—An interesting condition for the observation of sperm entrance is presented by this series of preparations and since the writer knows of no observations on sperm entrance in *Arbacia* some passing mention of it may be of interest. As may be seen from Fig. 4, two spermatozoa are in the act of entering an egg and many other such sections are to be found among the series. The egg has produced a small protrusion of protoplasm as an entrance cone that projects slightly above the egg surface; and within this cone lies the sperm head pulled out into a narrow chromatin band, one end of which projects down into the cytoplasm. No definitely formed fixation body, to which the inner end of the spermatozoön is attached, has been observed but the very shape and character of the entering spermatozoön would indicate a decided attraction from within the egg that acts upon the sperm head.

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[1] Normal sperm asters appear within five to ten minutes. Around most of the sperm heads is a small clear area produced by the retreat of protoplasmic granules of the egg from its periphery.

Fig. 4 was made from lot III Y^1 (page 283) and perhaps cannot be considered as representing the normal mode of entrance of the spermatozoön of *Arbacia* but it at least indicates the possible method of entrance. It may be necessary to attribute the appearance of the phenomenon to the general retarded condition of the egg protoplasm brought on as a result of the hypertonic treatment before insemination, but it would not seem impossible to discover a somewhat similar condition of sperm activity under normal conditions if the right stages were preserved in a close enough series. Penetration, in *Arbacia*, is effected much more readily than in *Nereis*, in which Professor Lillie[1] has illustrated so clearly the entrance of the spermatozoön. The most essential difference in the morphology of the process in the two forms is the absence of the attraction cone in *Arbacia* which in *Nereis* forms an attachment for the spermatozoön and apparently pulls the spermatozoön through a small aperture in the vitelline membrane as it recedes from the periphery of the egg. Immediately after the spermatozoön is taken inside the egg it assumes its normal appearance, for no elongated heads have been observed that were not in the act of entering the egg.

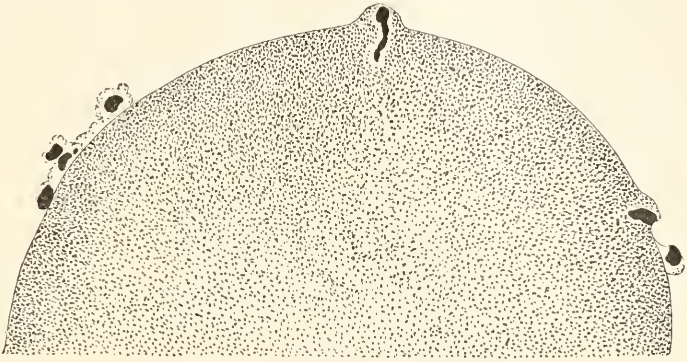

Fig. 4. × 3,000. Part of a section of an egg showing the entrance of the spermatozoön; one has practically completed its entrance and is rounding up, the other in the process of entrance. From same lot as Fig. 2.

3. *Insemination of Pure Cultures of Isolated Blastomeres.*

Since the experimental results of the writer did not agree with the views of Loeb—namely, that eggs whose development

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[2] See Lillie, F. R., '11 and '12.

had been initiated by an exposure to hypertonic sea-water, could yet be fertilized, produce fertilization membranes and develop into swimming larvæ, he was anxious to know if fertilization was prevented by failure of the sperm to enter or whether the individual blastomeres—the products of the first cleavage of an egg—had developed an immunity to the latent effects of a spermatozoön.

It is obvious that to determine this point from cytological preparations a pure culture of the first two blastomeres was highly desirable; and with considerable difficulty some two hundred and fifty or three hundred eggs that had segmented but once were isolated from lots of eggs that had been subjected to the weaker hypertonic solution. This pure culture of approximately five hundred half-eggs was heavily inseminated, allowed to stand for twenty minutes and preserved in Boveri's picro-acetic acid. A very few of these blastomeres had divided again into two daughter blastomeres (quarter-eggs) before the lot was inseminated, but every egg was isolated from the mass culture into a pure culture while in the two-celled stage.[1]

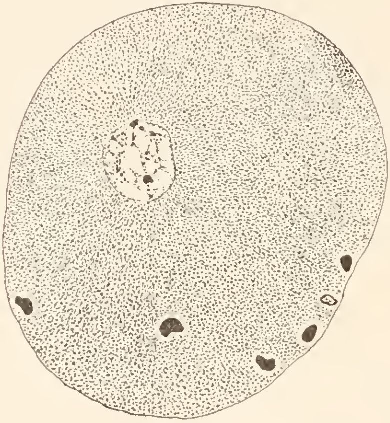

FIG. 5. × 3,000. Section of a quarter egg showing the presence of sperm nuclei. Weaker hypertonic solution, 30 to 40 minutes; egg segmented once, isolated, each blastomere segmented again. Killed 20 minutes after insemination.

Microscopical sections of these half-eggs prove to us beyond

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[1] As a definite membrane was not produced by the egg following the hypertonic treatment the blastomeres became entirely separated from each other in the pure culture. All were half-eggs when isolated.

any possible doubt that sperm do enter the blastomeres (Fig. 5) but the normal effect of a spermatozoön entering an egg is not apparent. Many of the sperm heads lie within the cytoplasm entirely unchanged, while others are seen to have increased in size from swelling, but most of these are undergoing evident degeneration, of which various stages can be seen (Fig. 6). The chromatin gradually loses its staining reaction, appearing much clearer than the unchanged sperm heads outside of the eggs, and some have so entirely lost their staining capacity that they appear only as clear vacuoles scattered throughout the cytoplasm of the half-eggs. Out of the vast numbers of sperm heads observed within these half-eggs only one was found that contained an indication of a good sperm aster. A few of the blastomeres are undergoing segmentation as a result of the hypertonic treatment and the spindles appear quite normal.

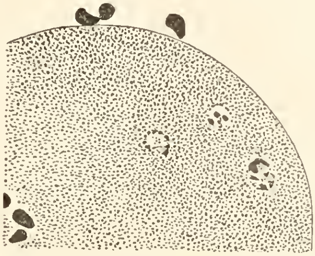

FIG. 6. × 3,000. Part of section of a quarter egg showing presence of both unchanged and degenerating sperm nuclei. No indication of fertilization changes. Same exposure as Fig. 5.

Here then the spermatozoön not only does not find a congenial environment in which normal reactions can go on, but the surroundings are even hostile and result in the disintegration of the spermatozoön without its having undergone any changes that indicate the least possibility of a fertilization reaction.

4. *Conclusions of Section IV.*

This cytological examination very clearly reveals the fact that the unfertilizable condition of eggs subsequent to an exposure to hypertonic sea-water is not due to the failure of a spermatozoön to enter the egg but that it is due to a change within the egg itself. Certain changes that are to be associated with initiation of development have produced an environment in which the reaction between a spermatozoön and an egg has been rendered impossible. *Spermatozoa enter the eggs in comparatively large numbers but the eggs are not fertilized.*

In the shorter exposure to hypertonic sea-water many eggs do react with a spermatozoön but since these are conditions in which a very small percentage of eggs segment and a still smaller percentage reach the swimming stage after the hypertonic treatment, the observations are interpreted as indicating an effect of the hypertonic solution below the optimum exposure for the initiation of development and as a consequence the eggs have experienced but little change from the normal condition; the exposure did not lead to the initiation of development, therefore the eggs still possess their capacity for fertilization. That this lack of a capacity for fertilization is one that is gradually developed as the exposure to the hypertonic solution is increased, is one of the factors that indicates the quantitative side of fertilization. After a short exposure, polyspermy, in which all sperm seem to have a part, is the general rule; a more pronounced effect of the hypertonic sea-water results in a greater number of sperm entering the egg, some showing very weak asters, others being only slightly swollen, while yet a more decided influence may create an environment within which the spermatozoön is not only inactive but also it may even undergo disintegration. Whatever changes have resulted from treatment with hypertonic sea-water, spermatozoa that gain entrance to eggs that have been exposed to optimum conditions for segmentation, or that have been decidedly over-exposed, do not meet with an environment that will permit of a reactivation of the egg.

This degeneration of spermatozoa in a non-reactive egg environment calls to mind essentially similar conditions, encountered normally, in the fertilization of meroblastic eggs such as the pigeon egg described by Blount ('07). Polyspermy in meroblastic eggs appears to be normal but the accessory spermatozoa, after the environment has been changed by reactions within the egg set up by a reaction of the egg with the successful spermatozoön, are repelled peripherally and degenerate and disappear.

V. Discussion.

For an egg to develop, some stimulus must initiate certain changes within it which, having been once set in motion, appear to be autonomous. If an egg is not fertilized, or its development initiated by some artificial agent, it normally does not develop. But studies in the field of the artificial initiation of development have brought to light many methods that may be employed successfully to set in motion the developmental mechanics latent within the egg. As yet we are unable to determine just the mechanism by which these agents are effective, but practically all methods agree in one essential at least—the permeability of the egg membrane is increased.

Practically every one is agreed that the egg must be considered, a very delicately adjusted physico-chemical system, within which, if a spermatozoön gains entrance, a characteristic series of reactions are set in motion that lead to development and a consequent change of the system. If artificial agents are employed as a substitute for the spermatozoön not only do we have conditions changed but also if the proper procedure is known and applied the end results are essentially the same as when initiation was effected by a spermatozoön, *e. g.*, cleavage, gastrulation and swimming larvæ. After the initiatory changes have been induced a spermatozoön in either case is entirely unable to produce any of its characteristic effects.

The delicately adjusted condition of the egg is only a temporary thing for if sea-urchin eggs are allowed to stand in sea-water for a day or more they gradually lose their power of fertilization. Not only can this unfertilizable condition, accompanied by increased permeability, be produced by standing but also by hypertonic sea-water, butyric acid, by a slight rise in temperature of the sea-water and several other agents including, of course, fertilization.

Many writers have considered an unfertilizable condition as an indication of the death of the egg but to cite only one instance to disprove this, the writer has called attention to the fact that if initiation of development is partially accomplished by a treatment with butyric acid and the membranes so formed are destroyed by shaking, sperm will enter the eggs of *Arbacia* but do not assert any capacity for the initiation of development: however if the eggs were subsequently exposed to the proper treatment with hypertonic sea-water a goodly per cent. will develop to swimming plutei. The non-fertilizable condition is not due to the death of the egg but to a changed physiological condition induced by the initiatory action of the butyric acid treatment. Whatever the changes brought about, all of the conditions mentioned above are accompanied by the loss of substances from the egg, if indeed the condition is not a result of the loss of some substance or substances that are necessary for fertilization. This loss may be due either to the escape from the egg of the necessary substance or to new chemical combinations within the egg made possible by the disturbance of its physiological equilibrium.

It is highly interesting to note that a qualitative and possibly a quantitative test for one substance at least has been discovered by F. R. Lillie. This substance fertilizin is continually liberated from the egg into the surrounding sea-water as long as it remains in a fertilizable condition and as pointed out on page 276 it has been found to afford an absolute test (using a fresh sperm suspension as an indicator) of the capacity of sea-urchin eggs for fertilization in seven analyzed cases and will probably be found to hold for all cases in *Arbacia*, as it is evidently an indicator of a generalized condition.

An analogous condition to that in *Arbacia*, of the loss of substances from the egg and the consequent lack of capacity for fertilization, has been found in other forms than the sea-urchin, though in no other forms than *Arbacia* and *Nereis* has a qualitative test for a substance been found. E. E. Just ('15) has discovered that if eggs from *Platynereis* are collected in sea-water they cannot be fertilized. This is due to some effect of the sea-water in causing the loss of necessary substances for fertilization either through diffusion from the egg or through new chemical combinations within the egg. If only a few drops of sea-water come in contact with the egg they will not fertilize despite the fact that sperm penetrate the eggs and produce weak changes characteristic of fertilization: the proper egg substances for the intiatory reactions are not available.

Miss Allyn ('12) found that any substance that had a tendency to initiate development in *Chætopterus* was detrimental to fertilization. She noted that the tendency was not for the exclusion of spermatozoa but rather for the production of polyspermy. The aberrant type of cleavage became more accentuated as the exposure to the initiatory agent was prolonged and would probably have entirely prevented fertilization with a slightly longer exposure. In this case the initiatory agents induced only a partial completeness of the reactions and as time went on fertilization became less and less possible, indicating the gradual loss of some substance whose presence is indispensable for fertilization. *Arbacia* eggs behave in essentially the same manner if over-exposed to butyric acid; polyspermy appears if insemination is carried out shortly after the exposure, but if the eggs remain standing in sea-water for a few hours insemination has no effect.

Just how hypertonic sea-water is effective we are not able to say, but the writer is inclined to the views of R. S. Lillie that a properly timed exposure to any substance that lowers permeability (unless it be decidedly toxic) allows of the combination of the substances within the egg that starts off the developmental reactions. If these changes are nearly enough the normal, development is quite complete up to certain stages in the life of the organism. The question, however, to which these experiments relate is the capacity for fertilization after activation by hypertonic sea-water.

The quantitative reaction idea has been pointed out quite freely in this and a preceding paper in considering the initiation of development, and certain instances of all phases of this process are to be had not only from the experiments themselves but also from the preserved material. From the purely parthenogenetic standpoint the ideas of the qualitative specificity of hypertonic sea-water as a factor in the initiation of development have been dealt a death blow by the experiments of R. S. Lillie ('15) on starfish eggs, and the purely quantitative idea substituted for the explanation. Lillie finds that not only can starfish development be initiated by a preliminary treatment with butyric acid followed by hypertonic sea-water, but also that a secondary treatment with butyric acid or warm sea-water or cyanide sea-water may be substituted for the hypertonic sea-water, or that both the preliminary and secondary treatments with warm sea-water may accomplish the same results. From inference any agent or combination of agents that cause a quantitative fulfillment of the reactions lead to the same end.

When a spermatozoön fertilizes an egg the optimum stimulation has been given to the egg, and we assume that the initiatory reactions have been quantitatively complete. But certainly in the initiation by artificial agents some of the eggs will not have been given the optimum conditions and will not have completed their reactions. If we can think in terms of chemical substances we will suppose that part of these have been left uncombined that are yet capable of some reaction with a spermatozoön if it but gain entrance to the egg. Consequently just so far as these fundamental reactions have been incomplete the spermatozoön is capable of asserting its latent stimulus for the activation processes. That fertilization is not complete in such cases is shown by the very irregular type of cleavage and the small per cent. of swimming larvæ and the poor quality of these, just as in a parthenogenetic treatment the same results are encountered if an exposure above or below the optimum one is given. If however the initiatory agent employed, has been affective enough to cause the production of good membranes (butyric acid), or has resulted in segmentation of the egg, the initiatory, reactive, egg substances appear to have been pretty well used up and so far as these experiments show there seems to be no tendency for a regeneration on the part of an egg of the fertilizable condition. The blastomeres of an egg appear to have no capacity, or at the most very little, to react with a spermatozoön. It has been pointed out above that out of hundreds of these isolated blastomeres never has one been able to produce a swimming larva, as a result of fertilization; and sections have revealed but one absolute case of sperm aster formation after penetration. One activation seems to preclude all others.

Fertilization appears to be due to an agent, spermatozoön, that initiates development by producing a condition that permits of the interaction of certain substances within the egg, which union curtails any further possibility of a subsequent fertilization. If the same conditions are produced by artificial parthenogenesis the same lack of the capacity for fertilization is evident.

Whether the lack of this capacity is due to the absence of one substance or of several substances, and whether the substance called fertilizin is the essential one or is only an indication of a certain physiological state of the egg is unknown. But in every case, so far examined, fertilizin has been present if the eggs could be fertilized and has been absent if there was no capacity for fertilization.

These results are weighty argument, if not definite proofs, against any idea of a sperm-borne substance that is necessary for the initiation of and the continuation of development. There is no evidence that the spermatozoön imparts a "lysin-like" substance that produces cytolysis within the egg and later carries into the egg a "corrective agent" to check the imposed cytolysis. The egg possesses all the substances it needs for development.

VI. Summary.

1. If *Arbacia* eggs are exposed to a weaker and a stronger concentration of sea-water for varying lengths of time a perceptible gradient of effectiveness from the entire absence of any reaction to an essentially normal parthenogenetic reaction is apparent.

2. The effective exposure is accompanied by a perceptible condition of increased permeability and substances escape from the eggs.

3. The superposition of insemination on the optimum hypertonic treatment does not increase the percentage of development.

4. Eggs when exposed to hypertonic sea-water for two hours usually cannot be fertilized; and in such a condition fertilizin cannot be detected by any known method. If however there yet remains a certain capacity for fertilization, fertilizin can be detected by its sperm agglutinating properties.

5. If *Arbacia* eggs are exposed to activating agents the permeability of the egg is increased, substances are lost from it and fertilization is impossible.

6. If eggs are sectioned, after insemination subsequent to an exposure to the weaker hypertonic solution, a gradient of activity of spermatozoa parallels, to a certain extent, the length of the exposure to hypertonic sea-water.

7. If eggs are sectioned after insemination, subsequent to an exposure to the stronger hypertonic solution, large numbers of eggs are seen to have several spermatozoa within their boundaries; but they usually do not exhibit any signs of activity; many are undergoing degeneration.

8. Sperm, in gaining extrance to an egg, are pulled out into a long thin chromatin band situated in the protoplasmic cone produced by the protoplasm of the egg.

9. If a pure culture of the first two cells, produced by cleavage, are inseminated, sperm enter, remain entirely quiescent, and may even undergo disintegration. They do not fertilize the blastomeres.

10. These experiments furnish no evidence for, but are contrary to, any view that a spermatozoön carries a substance into the egg that is necessary for development. There is no evidence of a sperm-borne "lysin-like" substance nor a secondary "corrective agent." The egg possesses all the essentials for development.

Hull Zoölogical Laboratories,
March 28, 1917.

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THE EFFECT OF STARVATION ON THE WING DEVELOPMENT OF MICROSIPHUM DESTRUCTOR.

LOUISE H. GREGORY.

As is the case with aphids in general, the life history of *Microsiphum destructor*, the green pea-aphid, shows a definite relation to the environment. Dimorphism is common. Winged and wingless individuals appear in varying numbers at different stages in the life history, the wingless forms predominating in the early part of the reproductive history, the winged forms increasing in number toward the end of the summer. While it is generally accepted that external conditions probably control the appearance of wings, very little data has been offered as proof. For a number of years, at the suggestion of Professor Morgan, I have experimented with the effect of starvation on the production of winged forms in the families of the green pea-aphid. I am glad to express my appreciation of Professor Morgan's helpful criticisms.

In starting all experiments, a single individual, selected to produce the stock family, was isolated on a young pea plant in a small pot and covered with a lamp chimney, the top of which was covered with gauze to prevent the escape of the aphid and the confusion of families. This method of isolation was used throughout the investigation and proved most successful, the plants growing normally, sufficient air entering at the top of the chimney. From the family of the stock aphid, some young were isolated and used as control lines, others were removed daily from the plant and starved for periods of different lengths, twenty-four hours, twelve hours and eight hours. Experience proved that fewer deaths resulted if the aphids were starved eight hours daily. After the period of fasting the starved aphids were isolated and their offspring observed. As will be clear later, the effect of starvation on wing development is not apparent in the individuals starved, but in their offspring.

TABLE I.

Starvation Exp. I.
Parent non-winged.
Both series from one parent.
A2 series younger.

Date 1913–14, winter.

Control.
1913–14.

No. Exp.	Hours Starved.	No. Non-winged Offspring.	No. Winged Offspring.	Total Family.	Per Cent. Winged.	Remarks.	No. Exp.	No. Non-winged Offspring.	No. Winged Offspring.	Total Family.	Per Cent. Winged.	Remarks.
A1 series	30					Starved—						*Winter*
No. 2		14	49	63		4 days—av. $7\frac{1}{2}$ h.	No. 2	31	14	45		
No. 3		6	34	40		per day—	No. 4	43	4	47		
No. 6		14	73	87			No. 6	28	23	51		
No. 7		25	61	86			No. 8	32	9	41		
No. 8		39	37	76			No. 10	41	3	44		
		—	—	—		Av. family		—	—	—		Av. family
		98	254	352	71+	70+		175	53	228	23%	45.4
A2 series	$32\frac{3}{4}$					4 days starved						
No. 2		30	0	30		av. 8.10 daily						
No. 3		27	23	50								
No. 4		34	40	74								
No. 5		23	54	77								
No. 6		24	43	67								
No. 7		35	43	78								
No. 8		16	20	36								
No. 9		25	26	51								
		—	—	—								
		214	249	463	53.8	Av. family 57						
Grand Total		312	503	815	61.1	Av. family 62						

The first experiments were carried on during the winter of 1913–1914 in the greenhouse at Columbia University (Table I.). Five young of a wingless mother were deprived of food 7½ hours daily for four days. These (A1 series) produced a total of 352 young, 254 or 71 per cent. of which were winged. In the second series (A2) younger members of the same family, 8 aphids were starved 8 hours daily for four days. The total number of offspring in the eight families was 463, of which 249 or 53 per cent. were winged. In the control series of a total of 228 (young of five families) 23 per cent. were winged showing a difference of 48 per cent. if compared with the A1 series and of 20 per cent. with the A2 series. If the entire A series is considered as a whole, among the young of 13 families 61 per cent. were winged. Apparently the lack of food has had its effect on the second generation, none of the starved individuals developing wings.

The experiments were repeated at Woods Hole in the summer of 1915 (Table II.). Again the young of a wingless mother were used for the experiment and control. The families of seven wingless females starved 45 hours, were observed and out of a total of 188 young, one winged form only appeared. The seven families of the control series showed 3 winged individuals. Apparently the effect of fasting was overcome in some way.

Considering that the atmospheric conditions had been unusually favorable for the development of the peas, the weather having been very hot with much humidity, I concluded the food had been sufficiently rich to overcome any adverse effects of starvation, and that in order to induce the effects of starvation, it would be necessary to increase the number of starvation periods. Consequently the experiments were repeated the following summer in Princeton, Mass. (Table III.).

Five young starved 60 hours produced a total of 254 offspring, 30 per cent. of which were winged. Ten individuals forming the control series produced 514 young, of which 56, or 9.7 per cent., were winged. Here again starvation has had its effect.

Summarizing all the experiments on wingless parents, it is seen in Table III., that 25 starved individuals produced 1,257 young, 46 per cent. of which had wings: that 22 normal indi-

TABLE II.

1915. *Starvation Exp. II.* Parent non-winged. 1915. *Control.* Parent non-winged.

No. Exp.	Hours Starved.	No. Non-winged Offspring.	No. Winged Offspring.	Total Family.	Per Cent. Winged.	Remarks.	No. Exp.	No. Non-Winged Offspring.	No. Winged Offspring.	Total Family.	Per Cent. Winged.	Remarks.
A_2	45					Woods Hole	A_1					Woods Hole
(1)		37	0	37		Plants luxuriant	(1)	38	0	38		
(3)		15	0	15			(2)	71	3	74		
(4)		18	0	18			(3)	49	0	49		
(5)		32	0	32			(4)	39	0	39		
(6)		36	0	36			(5)	48	0	48		
(7)		18	0	18			(6)	34	0	34		
(10)		31	1	32			(10)	65	0	65		
		—	—	—				—	—	—		Av. family
		187	1	188		Av. family 26.8		344	3	282	1.	39.5

viduals produced 1,149 young, 9.7 per cent. of which were winged. The starved mothers had about three times as many winged young as the control. Since all the families were subjected to the same conditions of temperature and humidity, the variation in the results must be due to the only factor in which they differ, namely the food supply.

Temperature undoubtedly may effect the production of wings as Ewing[1] has shown in his experiments with *Aphis avenæ*. He found that by keeping the temperature constant at 65° F., he could prevent the appearance of any winged forms. At lower and higher temperatures winged forms appeared. These results might be interpreted as due *not* to the direct effect of temperature on the development of wings but on the growth of the plants or the food supply and thus indirectly affecting the metabolism of the individuals.

If the summer experiments are compared with those in the winter, it will be observed that the percentage of winged forms is less in both starved and control lines. However the percentage of increase in number of winged forms in the starved lines remains constant. In the summer as in the winter there are three times as many winged young in the starved lines as in the control. I see no reason why the temperature might not have been effective indirectly in the lessening of the wing production in both starved and control summer lines. The atmosphere of the greenhouse was practically constant, while the summer experiments were carried on out of doors and were subject to all the daily changes in temperature and moisture. The actual difference in the development of wings at any one time must have been due to the difference in food supply, temperature having no part. I believe that if aphids were starved for long periods and kept at a constant temperature of 65° F., Ewing's optimum temperature, winged individuals would appear.

Overcrowding on the plants has been suggested as a possible cause of wing development. In nature this might be true, the results however being due to a lessening of the food supply. In my experiments there was no overcrowding as the young were

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[1] Ewing, H. E., " 87 Generations in Parthenogenetic Pure Line of *Aphis avenæ*," Biol. Bull., Vol. XXXI., No. 2, 1916.

TABLE III.

Starvation Exp. III. 1916 July. Parent non-winged.

Control. 1916 July. Parent non-winged.

No. Exp.	Hours Starved.	No. Non-winged Offspring.	No. Winged Offspring.	Total Family.	Per Cent. Winged.	Remarks.	No. Exp.	No. Non-winged Offspring.	No. Winged Offspring.	Total Family.	Per Cent. Winged.	Remarks.
	60 hrs.					Princeton, Mass.						
1		35	51	86		Alt. 1100 ft.	2	47	0	47		
3		23	11	34		Atmosphere dry	4	56	3	59		
5		50	1	51		and cool	6	33	5	38		
9		39	14	53		Experiments in	8	63	0	63		
11		29	1	30		open	10	61	0	61		
		—	—	—			12	58	0	58		
		176	78	254	30.7	Av. family 50+	14	79	11	90		
							16	37	35	72		
							18	42	0	42		
							20	42	2	44		
								—	—	—		
								518	56	574	9.7	Av. family 57.

Summary

No. Exp.	No. Non-winged Offspring.	No. Winged Offspring.	Total Family.	Per Cent. Winged.	Remarks.	No. Exp.	No. Non-winged Offspring.	No. Winged Offspring.	Total Family.	Per Cent. Winged.	Remarks.
1913–14 13 fam.	312	503	815	61.7	*Winter* Starved 30–32 hours. Av. family 62.	1913–14 5 fam.	175	53	228	23	*Winter* Av. family 45.
1915 7 fam.	187	1	188		*Summer* Woods Hole Starved 45 hours.	1915 7 fam.	344	3	347		*Summer*
1916 5 fam.	176	78	254	30.7	*Summer* Princeton Starved 60 hours.	1916 10 fam.	518	56	574	9.7	*Summer*
	—	—	—				—	—	—		
Total 25 fam.	675	582	1257	46.3	Av. fam. 50.2	Total 22 fam.	1037	112	1149	9.7	Av. fam. 52.2

TABLE IV.

1913–14.	*Starved.* Parent winged.					1913–14.	*Control.* Parent winged.				
No. Exp.	No. Non-Winged Offspring.	No. Winged Offspring.	Total Family.	Per Cent. Winged.	Remarks.	No. Exp.	No. Non-winged Offspring.	No. Winged Offspring.	Total Family.	Per Cent. Winged.	Remarks.
Winged A^1 4	65	2	67	.3	Starved 30 h.	1	15	0	15		
	—	—	—			3	26	0	26		
	65	2	67			5	25	4	29		
						7	28	0	28		
						9	27	0	27		
							—	—	—		
							121	4	125	3.	Av. family 25.
1914–15.											
Winged G (5).	29	0	29	0	♀ died young.	No.					
(6).	15	0	15		Starved 45 h.	controls					
	—		—								
	44		44								
Non-winged—											
G (2)	11	8			" "	"					
(8)	44	16									
(9)	14	15									
	—	—									
	69	29	98	29.							

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removed from the plants as soon as it was possible to determine the presence or absence of wing buds.

It has been suggested that young produced in the early part of the reproductive life of the mother would be better nourished and fewer wings would develop than among those born at the end of the period of reproduction. A consideration of the A1 and A2 series of Table I. seems to disprove this, as more wings developed in the families of the older members.

It might also be suggested that the effect of starvation was to reduce the size of the families and consequently the percentage of winged forms might be raised. In some cases the size of families of the starved mothers was smaller than that of the control, in other cases it was greater. The average sized family was 50.2 in the starved lines and 52.2 in the controls. The difference is not large enough to be important.

A few experiments were carried on with starved winged mothers. These are difficult to obtain, since the number normally produced is low and since it is impossible to distinguish wing buds at the early period when starvation is begun, thus the chances for finding winged forms among the starved young are slight. The control experiments (Table IV.), show that of the young of five winged mothers only 3 per cent. were winged. In the few families of starved winged mothers the percentage is less, often none of the young develop wings In passing it might be noted that in the G series (Table IV.) no. 5 and no. 6 winged individuals starved 45 hours produced no winged young, while their wingless sisters nos. 2, 8, 9, produced 29 per cent. winged forms. Apparently starvation has little effect on winged mothers. Teleologically this result would be expected. There would be no need for more winged forms in nature, since the parent had migrated to a fresh food supply.

In conclusion it seems most probable that the lessening of the food supply is the primary factor in determining the development of wings in the offspring of wingless mothers. The wing anlage appears to be present in all the parthenogenetic females and depends directly upon the condition of the food supply of the mother for its stimulation or suppression of development.

Columbia University,
June, 1916.

Vol. XXXIII. November, 1917. No. 5.

BIOLOGICAL BULLETIN

THE PRODUCTION OF FUNCTIONAL AND RUDIMENTARY SPERMATOZOA IN ROTIFERS.[1]

D. D. WHITNEY.

Marked dimorphism in the spermatozoa of a few animals has been briefly recorded by several observers, but no detailed study has been made of such forms to determine how the two kinds of spermatozoa are formed nor has there been offered an explanation as to their probable functions. While looking at the plates in "The Rotifera," by Hudson and Goss, I noticed sketches of the two dimorphic spermatozoa of the marine rotifer, *Brachionus mulleri*. As this rotifer occurs in countless numbers in a salt-water lake near Lincoln, Neb., an excellent opportunity was offered to reëxamine it in an attempt to find an explanation for the production of the two kinds of spermatozoa. After reëxamining the spermatozoa of this rotifer and confirming the observations of Hudson and Goss the spermatozoa of eight other species of rotifers were examined and were found also to be composed of two kinds. Thus indicating that this phenomenon of dimorphism of spermatozoa may be general for all the species of the whole group of rotifers which produce male individuals.

The technique of examining the spermatozoa is very simple. Mature free swimming males were placed on a slide in a drop of the culture water and covered with a cover glass. Then by pressure the males were forced to eject the contents of the single testis into the water. The two kinds of spermatozoa could be readily seen even with the low powers of the microscope and were as easily counted. Early stages in the development of the spermatozoa as far back as the early spermatids were studied by crushing the eggs containing the unhatched immature males. All stages of development from the earliest spermatid up through to a mature spermatozoön were easily seen, especially in the species of rotifers whose males form only a few spermatozoa.

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[1] Studies from the Zoölogical Laboratory, The University of Nebraska, No. 117.

Brachionus mulleri, the salt-water rotifer, was the first species examined and because of the relatively small number of spermatozoa produced by the males it proved to be the most favorable form in which to see the early stages in the development of the spermatozoa as well as for making accurate counts. The motile spermatozoa were extraordinarily large and measured about one third the total length of the male individual that produced them. Probably on account of the large size of the spermatozoa a male is physically unable to form very many. These motile spermatozoa were long and somewhat slender with a pointed anterior end. The posterior two-thirds of the spermatozoön is very motile and has a prominent, undulating, median, and dorsal membrane which aids greatly in the locomotion. The sketch of a mature spermatozoön in Fig. 1, *E*, is a dorsal view and does not show adequately this membrane. These spermatozoa swim freely about in the water for a short time after they are forced out from the body of the male.

The spindle-shaped spermatozoa are incapable of any locomotion. They are uniform in shape and never change their form. They are very much smaller than the motile spermatozoa. Fig. 2, *D*, shows several of these. As the tissues of the males are very transparent both kinds of these spermatozoa may be readily seen inside the testis. The spindle-shaped spermatozoa are usually found collected together near the posterior end of the testis, although sometimes only a few are thus collected together while the remainder are scattered throughout the testis among the motile spermatozoa.

When the immature males are crushed the spermatids still united in masses separate readily from the general debris. Various stages in the development of the spermatids and spermatozoa may be seen in accordance with the stage of development of the male. All spermatids or immature spermatozoa in each male are generally found in the same stage of development.

The relative number of the two kinds of spermatozoa varied widely when counts were made from the mature free swimming males taken from a general culture of rotifers. It is very prob-

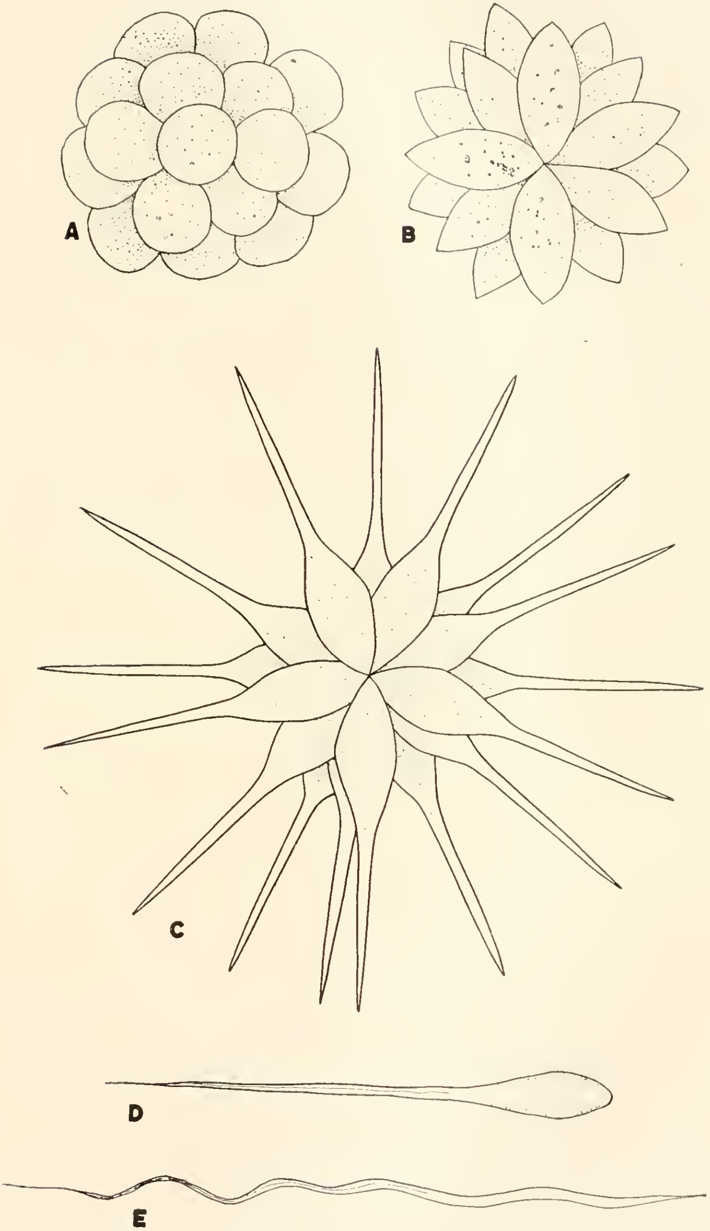


FIG. 1. Formation of the functional spermatozoa in *Brachionus mulleri*. *A*, young spermatids; *B*, older spermatids; *C*, young immature spermatozoa; *D*, older immature spermatozoön; *E*, mature, motile, and functional spermatozoön.

able that many of these males had already paired with females and consequently had shed some of their spermatozoa. This would explain the variability of the spermatozoa counts in the first twenty-one males recorded in Table I.

Table I.

Brachionus mulleri. Showing the relative proportional number of the two kinds of spermatozoa found in each testis of thirty male individuals. The rudimentary spermatozoa were never found as numerous as the functional spermatozoa in any testis of a young male. In the immature males in which none of the spermatozoa had been fully formed or shed the larger spermatids that ultimately develop into the functional spermatozoa were found to be exactly twice as numerous as the smaller spermatids that ultimately develop into the rudimentary spermatozoa.

Serial Number.	State of Development of Males.	Number of Functional Spermatids or Spermatozoa in Testis.	Number of Rudimentary Spermatids or Spermatozoa in Testis.	Serial Number.	State of Development of Males.	Number of Functional Spermatids or Spermatozoa in Testis.	Number of Rudimentary Spermatids or Spermatozoa in Testis.
1	Mature	50	26	16	Mature	5	0
2	"	48	—	17	"	24	8
3	"	—	12	18	"	9	10
4	"	—	0	19	"	28	10
5	"	6	2	20	"	18	6
6	"	—	15	21	"	60	25
7	"	2	1	22	Immature	38	18
8	"	20	11	23	"	70–80	38–40
9	"	—	18	24	"	40	18–20
10	"	22	13	25	"	40	18–20
11	"	4	0	26	"	60	32
12	"	15	5	27	"	64	32
13	"	56	35	28	"	40	20
14	"	28	11	29	"	56	28
15	"	40	22	30	"	48	24

When immature males were crushed one could readily count all the spermatids of both kinds and could definitely determine the relative number of each. It was seen at once when all spermatids were counted that the two kinds of spermatids that develop into the two kinds of spermatozoa did not occur in equal numbers. The large spermatids which ultimately develop into the large motile spermatozoa were found to be exactly twice as numerous as the smaller spermatids which ultimately develop into the small spindle-shaped spermatozoa. This is clearly shown in the last few males recorded in Table I. Figs. 1 and 2 show a series of the various stages of development from early spermatids to mature spermatozoa of both kinds of these dimorphic spermatozoa.

Mature males of *Hydatina senta* were crushed and examined and were found also to possess the two kinds of spermatozoa as markedly dimorphic as those in *Brachionus mulleri.* Many more spermatozoa are formed in the testis and consequently the counts could not be as readily or as accurately made as in *Brachionus mulleri.* Counts enough, however, were made to show that the large motile spermatozoa were about twice as numerous as the smaller immotile spindle-shaped spermatozoa.

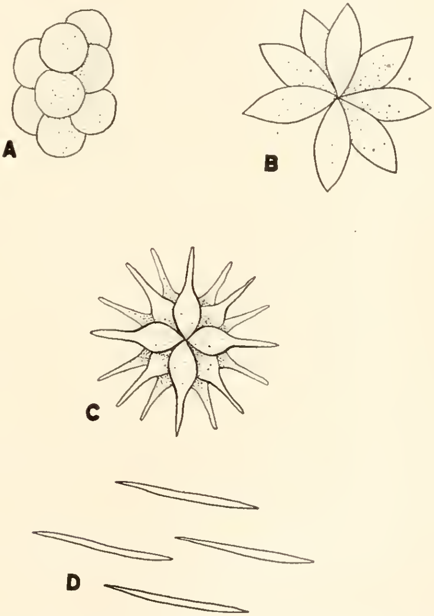


FIG. 2. Formation of the rudimentary spermatozoa in *Brachionus mulleri.* *A*, young spermatids; *B*, older spermatids; *C*, young immature spermatozoa; *D*, mature, immotile, and rudimentary spermatozoa.

The counts are shown in Table II. and drawings of the two kinds of spermatozoa are shown in Fig. 5.

The spermatozoa were examined next of the large viviparous rotifer, *Asplanchna amphora*, and although no very accurate counts could be made the two kinds of spermatozoa were conspicuously present. The highest count for the motile spermatozoa was about 200, while the highest count of the immotile spindle-shaped spermatozoa was about 100. These counts were made from males which were isolated at birth before they had had an opportunity to pair with females and to shed their spermatozoa. They show that the motile spermatozoa are about twice as numerous as the immotile kind. Drawings and counts of these two kinds of spermatozoa are shown in Fig. 3 and Table III.

TABLE II.

Hydatina senta. Showing the relative proportional number of the mature dimorphic spermatozoa found in each testis. In general the large motile spermatozoa were twice as numerous as the small spindle-shaped spermatozoa.

Serial No.	State of Development of Males.	Number of Functional Spermatozoa in Testis.	Number of Rudimentary Spermatozoa in Testis.	Serial No.	State of Development of Males.	Number of Functional Spermatozoa in Testis.	Number ot Rudimentary Spermatozoa in Testis.
1	Mature	60	18	8	Mature	—	50–60
2	"	—	24	9	"	50–60	25–30
3	"	72	26	10	"	0	5
4	"	160–200	—	11	"	20	18
5	"	130	44	12	"	186	—
6	"	—	40	13	"	90	52
7	"	—	30				

TABLE III.

Asplanchna amphora. Showing the relative proportional number of the mature dimorphic spermatozoa found in each testis. In general the large motile spermatozoa were twice as numerous as the small spindle-shaped spermatozoa.

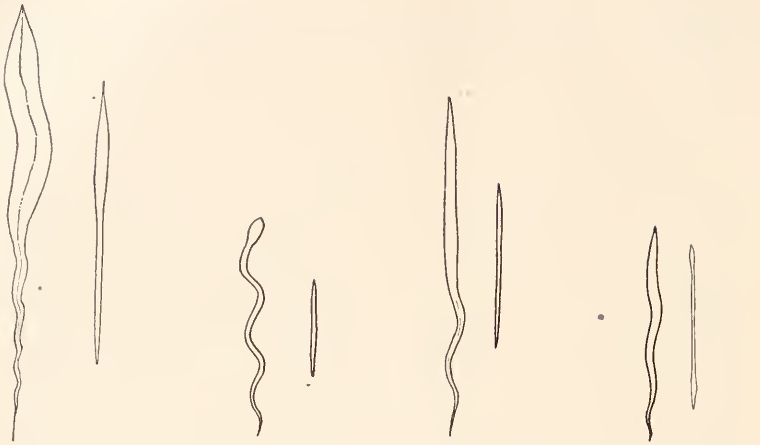
Serial Number	State of Development of Males.	Number of Functional Spermatozoa in Testis.	Number of Rudimentary Spermatozoa in Testis.
1	Mature	200	50
2	"	200	60
3	"	—	104
4	"	160	75

In addition to the spermatozoa of these three species of rotifers already described the spermatozoa of six other species of rotifers were carefully examined. These species were *Polyarthra platyptera*, *Diglena catellina*, *Euchlanis dilatata*, *Metopidia lepadella*, *Brachionus urceolaris* and *Brachionus bakeri.* In all cases the two kinds of spermatozoa were found and the larger motile ones were more numerous than the smaller immotile ones. Drawings of these spermatozoa are shown in Figs. 4, 6, 7, 8, 9 and 10.

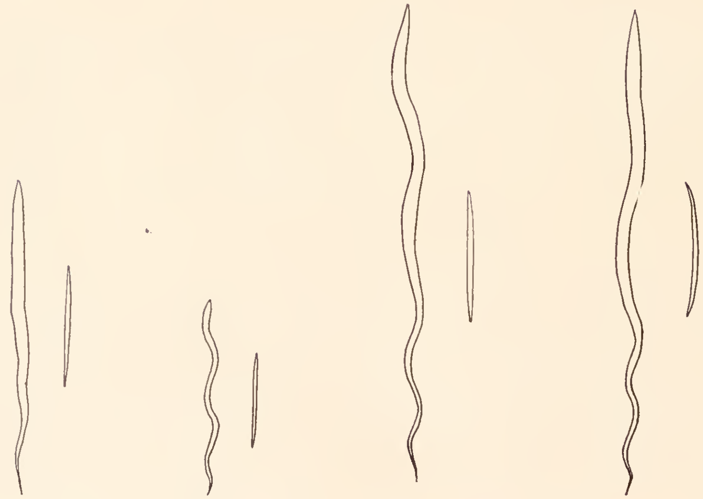
Morgan has found in the study of the spermatogenesis of some of the phylloxerans that the secondary spermatocytes are of two kinds—the normal and the rudimentary. The normal spermatocytes divide again to form the spermatids, each of which develops later into a functional spermatozoön. The rudimentary spermatocytes do not divide again and do not form spermatids or spermatozoa. They merely persist for a time as rudimentary secondary spermatocytes and then finally disintegrate and disappear. Morgan has also found the same phenomenon in some of the aphids, Stevens and von Baehr have also found it in the aphids, Meves has found it in *Vespa germanica*, Mark and Copeland have found it in *Vespa maculata*, and Lams has found it in an ant, *Campodinotus herculaneus*.

In the grand total count of all the spermatocytes and spermatids at the end of the final spermatocyte divisions in all of these forms enumerated in the preceding paragraph the normal spermatids should have been twice as numerous as the rudimentary spermatocytes. No counts, however, were made and consequently this point was not settled by this method but it was demonstrated in another manner by showing that only one spermatocyte division occurred in the rudimentary spermatocytes and two divisions occurred in the normal spermatocytes in the process of formation of the normal spermatozoa. In 1909 Morgan said in regard to the rotifer, *Hydatina senta*, which at the time had not been investigated. "Theoretically there should not be two spermatocyte divisions but only one true division. It remains to be seen whether this prediction proves true."

The rotifers have not only fulfilled but have exceeded Morgan's expectations! They not only produce normal spermatids and rudimentary secondary spermatocytes in the ratio of 2:1 as probably do these forms already mentioned, but they develop both of these into spermatozoa which are later shed by the males. Those of one kind are large, motile and probably functional while those of



3. ASPLANCHNA AMPHORA 4. POLYARTHRA PLATYPTERA 5. HYDATINA SENTA 6. DIGLENA CATELLINA



7. EUCHLANIS DILATATA 8. METOPIDIA LEPADELLA 9. BRACHIONUS URCEOLARIS 10. BRACHIONUS BAKERI

FIGS. 3–10. Dimorphic spermatozoa of eight species of rotifers showing the larger, motile, and functional spermatozoa and the smaller, immotile, and rudimentary spermatozoa. In all of these species the drawings were made of spermatozoa that were taken from mature free-swimming males except in Fig. 4 which were made of spermatozoa of unhatched immature males. All were drawn to same scale as Figs. 1–2.

the other kind are small, immotile and probably rudimentary, and without function. The rudimentary spermatozoa must necessarily develop directly from the secondary spermatocytes without the occurrence of the second spermatocyte division in order to form the ratio of two normal spermatozoa to one rudimentary spermatozoön. If they developed from rudimentary spermatids which were formed by the division of the secondary spermatocytes the ratio would be one functional spermatozoön to one rudimentary spermatozoön. This interpretation of the observed ratios would therefore place the rotifers in the same category as the other animals already mentioned as far as the formation of normal spermatids and of rudimentary secondary spermatocytes are concerned. They would, however, have this point of difference, viz.: that the degree of degeneration of these rudimentary secondary spermatocytes has not progressed as far as to render such spermatocytes incapable of developing directly into degenerate spermatids and spermatozoa of a certain kind, as is the case of the other forms studied.

In the mature parthenogenetic egg that develops into the male individual there is the reduced number of chromosomes. This contains one half as many chromosomes as are found in the parthenogenetic egg that develops into the female. This fact was made out in *Hydatina senta* but the exact number of chromosomes occurring in each kind of egg has never been determined. If the male in the beginning has already the reduced number of chromosomes no further reduction of chromosomes is necessary in the formation of the spermatozoa. The race, however, may have retained the former ancestral process of performing the first spermatocyte division, regardless of the results, in the formation of the secondary spermatocytes but as there is no general reduction of chromosomes to be made at this stage presumably none occurs. One half of the secondary spermatocytes very likely contain fewer or perhaps no chromosomes at all and consequently are very degenerate and rudimentary and without a second division develop directly into degenerate spermatids and spermatozoa.

The male parthenogenetic eggs are the ones that are fertilized as has been determined by Maupas, Lauterborn, Whitney, and

Shull and after fertilization always develop into females but never into males. Thus the fertilization causes a change of the sex in the resulting individual from such eggs. All of the functional spermatozoa must therefore be identical in their influence in producing females from eggs that if unfertilized would otherwise have produced males.

Summary.

1. Dimorphic spermatozoa are produced and shed by the rotifers, *Brachionus mulleri*, *Asplanchna amphora*, *Polyarthra platyptera*, *Hydatina senta*, *Diglena catellina*, *Euchlanis dilatata*, *Metopidia lepadella*, *Brachionus urceolaris* and *Brachionus bakeri*.

2. One class of the spermatozoa is composed of large, vermiform, and motile spermatozoa and the other class is composed of small, spindle-shaped and immotile spermatozoa.

3. The number of motile spermatozoa produced is exactly twice the number of immotile spermatozoa produced.

4. The motile spermatozoa probably develop from the cells formed by the second division of the normal spermatocytes but the immotile spermatozoa probably develop directly from the rudimentary cells formed by the first division of the spermatocytes.

5. The motile spermatozoa are probably functional but the immotile spermatozoa are probably rudimentary and functionless.

6. The functional spermatozoa are all identical in their power of determining the sex of the individual that develops from a fertilized egg. After a functional spermatozoön has fertilized a parthenogenetic male egg the egg eventually develops into a female individual instead of developing into a male individual as it otherwise would have done if it had not been fertilized.

Zoölogical Laboratories,
The University of Nebraska,
Lincoln, Nebraska,
June 22, 1917.

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November 3.—In some recent and more careful studies on the spermatogenesis of *Brachionus mulleri* it has been determined that the bodies which have been designated as normal spermatozoa are only the detached tails of these spermatozoa and are not the complete cell. When the males are compressed under a cover glass the tails become detached from the heads of the spermatozoa while inside the body of the males and then are immediately extruded and remain in an active state for a considerable length of time. This fact was not observed by Hudson and Goss as is shown by their plate 27 nor by myself until very recently.

As these motile tails resemble very closely the motile spermatozoa of the other species of rotifers described in this paper it is very probable that all the motile and supposedly complete spermatozoa observed were also only tails. Whether the bodies that were considered rudimentary spermatozoa are only tails of such spermatozoa or are complete spermatozoa has not been definitely decided.

These new detailed facts do not, in the least manner, invalidate the main issue of the paper in regard to the two classes of spermatozoa and their ratio. Additional observations and data derived from these recent studies will be published shortly.

THE AUTHOR.

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THE SPERMATOCYTIC DIVISIONS OF LEPTOCORIS HÆMATOLOMA.[1]

LELIA T. PORTER.

INTRODUCTION.

The spermatogenesis of *Leptocoris hæmatoloma* (family Coreidæ) very closely resembles that of *Anasa tristis*, as described by Paulmier. During the past few years it has been used extensively here in the course on cytology to demonstrate the behavior of the X-chromosome. As a matter of record, it seems worth while to give a very brief account of the spermatocytic divisions. Furthermore, the material is very fine for the study of tetrad formation, and it is hoped later to publish an account of this process.

The material on which these observations were made was collected in November and December, 1914, on the plant *Cardiospermum halicacabum*, the balloon vine which is very common in this vicinity. It may be of interest, however, to know that this is the only plant on which we have found this bug. The testes, which are large yellow pear-shaped bodies located at the base of the third pair of walking legs were dissected out in Ringer's solution and fixed immediately in Bouin's fluid. They were stained with iron hæmatoxylin and counterstained with eosin and light-green. Other fixing agents were used, but this proved to be the best.

The material is excellent for the study of spermatogenesis, as the chromosomes are not numerous, and vary greatly in size. It is possible to follow accurately the courses of the smallest, largest and accessory chromosomes throughout their entire history.

The spermatogonial complex of *Leptocoris hæmatoloma* consists of thirteen chromosomes (Figs. 1 and 2). These vary in size, but can be classified in three groups. The members of two pairs are quite large, three pairs are of medium size, while the sixth pair and the accessory chromosome are rather small. On account of their size and shape, one has little difficulty in recognizing the members of the various pairs.

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[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 138.

After a series of successive spermatogonial divisions, the thirteen chromosomes pass through the characteristic synaptic and growth stages, giving rise to six tetrads and one dyad, typical of the primary spermatocyte. These tetrads vary greatly in size, the largest and smallest being easily recognized in Figs. 3 and 4. Three of the tetrads approximate each other very closely, and the remaining one is slightly smaller than the largest. The dyad representing the accessory chromosome lies close to the nuclear wall in Figs. 3 and 4, but I have no reason to believe that this is necessarily characteristic. Figs. 3 and 4 were selected because in each the entire number is seen, though scattered irregularly throughout the nucleus. At this stage the nuclear membrane is intact and the cell itself has increased enormously in size.

The tetrads are then drawn into the central plate and there is no question as to their final arrangement Fig. 5 shows the typical position of the various chromosomes in the central plate of the first spermatocyte. I have examined numbers of cells and at this stage have always found the six chromosomes arranged in a ring with the smallest in the center. The accessory chromosome always lies in the circle. Paulmier has found this to be the case in *Podisus*, *Lygæus* and *Chariesterus*, but Henking finds no such peculiar arrangement in *Pyrrochoris*.

First Spermatocytic Division.

The spindle of the primary spermatocyte is ordinarily rather broad but as the chromosomes begin to pull apart, it becomes in many cases, long and slender. The fibers of the spindle converge at the poles, but no centrosomes could be found in any of these stages. This may have been due to the stains used. The chromosomes in the metaphase plate do not divide synchronously. The accessory tends to divide precociously (Figs. 8 and 9) and is generally followed by the smallest chromosome (Figs. 6 and 7). As the accessory advances toward the poles, the larger chromosomes are seen to be pulled apart. As they divide and separate they are usually still connected by two thick threads (Figs. 6, 7, 9). These fibers stain deeply with hæmatoxylin but are lost sight of as the chromosomes are pulled farther apart and the cell wall begins to constrict. There is no sign of a zwischenkörper as described by Paulmier for Anasa. Each of the two resulting secondary spermatocytes (Figs. 11, 12, 13) contains six chromosomes in the form of dyads and one monad, the accessory. There is no trouble identifying the accessory because of its size and position.

SECOND SPERMATOCYTIC DIVISION.

The second division follows directly upon the first without any resting stage. In the equatorial plate of the second division the chromosomes are arranged in the characteristic ring of six with the smallest chromosome in the center. In the case of *Anasa tristis* Paulmier finds no such regular arrangement though he says the small chromosome generally lies in the center. The chromosomes now arrange themselves on the middle of the spindle and very soon the accessory is seen to leave the group and pass undivided in advance of the others toward one of the poles (Figs. 14, 16, 17, 18, 19, 20). In *Anasa* the accessory lags, and is not drawn toward one of the poles until the other chromosomes have practically come to the end of their movement. The remaining dyads then divide apparently in the same order as for the first division, the smallest leading (Figs. 15, 17, 19). These then pass to opposite poles and the cell constricts in the middle. The resulting cells are smaller than those of the first division, half of them containing seven chromosomes, and half six (Figs. 21, 22, 23, 14). The chromosomes of these spermatids have the same characteristic arrangement as those of the primary and secondary spermatocytes, one half of them having a ring of six with a small one in the center and the other a ring of five with the small one also in center. There is a typical massing of the chromosomes in the spermatid before the nuclear wall is formed.

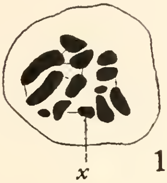
The spindle of the second spermatocyte is similar in shape to that of the first, being rather broad and thick at the initiation of division, and as the chromosomes are pulled apart, taking on a slender elongated appearance.

In conclusion it may be suggested that the female has two X-chromosomes, or fourteen in all. Wilson reports that *Leptocoris trivittatus* has fourteen in the female and thirteen in the male.

Explanation of Plate I.

All drawings were made with the aid of a camera lucida at table level. A Zeiss microscope with compensating 12 ocular and 2 mm. oil immersion objective was used. The drawings have been reduced one third off.

1, 2. Equatorial plate of spermatogonia.
3, 4. Tetrad stages, showing all chromosomes.
5. Polar view of first spermatocyte.
6–8. Side view of same.
9. Early anaphase of first division.
10. Side view of first spermatocyte division.
11–13. Polar view of second spermatocyte.
14–20. Second spermatocytic division.
21, 22. Spermatids, corresponding daughter cells.
23, 24. Spermatids, corresponding daughter cells.



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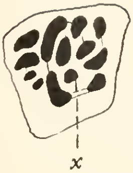

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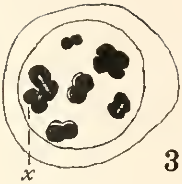

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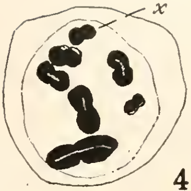

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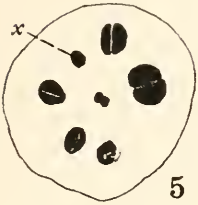

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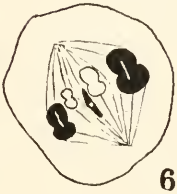
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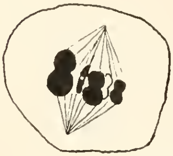
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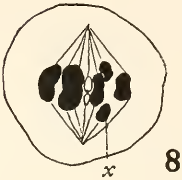

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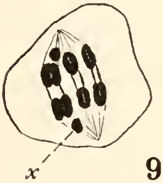

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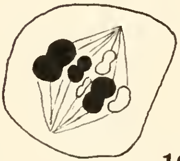
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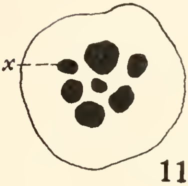

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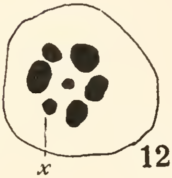

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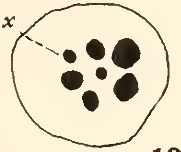

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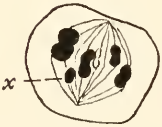

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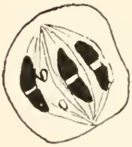
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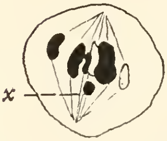

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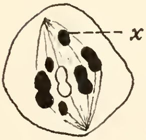

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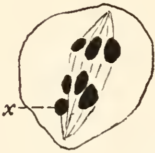

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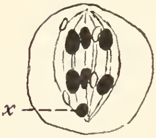

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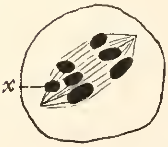

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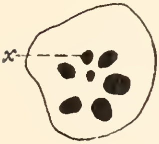

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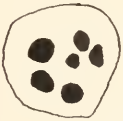
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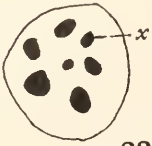

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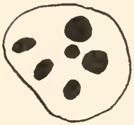
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LELIA T. PORTER.

THE FATE OF THE UNFERTILIZED EGG IN THE WHITE MOUSE

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It has long been known that in mammals many more eggs are ovulated than develop into embryos, due in part to the failure of the spermatozoa to reach them, and perhaps more often to the inability of the eggs to implant themselves in the uterus. Such eggs are said to degenerate, but the story of their fate has been recorded in only a few instances. Since ovulation in the mouse is independent of copulation, this animal was selected for the investigation. It is natural to suppose that the process would be similar to the degeneration of the ovarian egg which has been prevented from leaving the ovary, the process termed follicular atresia.

The atresia of ovarian eggs in mammals has been described in detail by many investigators, but the degeneration of the unfertilized egg in the Fallopian tube and uterus has been given but little special study. Heape ('05) working on the domestic rabbit, mentions the finding of eggs in the tube which for some reason or other had not been fertilized and were in a process of degeneration. Hartman ('16), in his study of the early development of the American opossum, also refers to the great number of unfertilized eggs, approximately 50 per cent., which he found in the uterus, in an earlier or later stage of degeneration. He ventures the opinion that these eggs would eventually leave the uterus.

Students of atresia have usually recognized that there is an early disturbance of the nucleo-cytoplasmic relationship, although they have not always given it that name, and that it was followed by a rearrangement of the cytoplasm. Some writers however insist that a true parthenogenetic cleavage may take place, and continue in a more or less normal manner up to, in some cases, the ten or twelve cell stage before the degenerative processes get the upper hand.

The purpose of the present investigation is to describe step by step the degeneration, whether it be fragmentation or parthenogenetic development, of the unfertilized egg in the white mouse, and to compare the process with follicular atresia in the mouse and other mammals.

The work was undertaken at the suggestion of Prof. W. R. Coe, and done under his supervision. I am pleased to acknowledge his many valuable suggestions during the course of the investigation.

LITERATURE.

Kingery ('14) describes the method of atresia in the follicles of young mice. In his opinion the process is one of degenerative fragmentation only, that the spindle fibers of the second maturation spindle break, forming aster-like radiations and that later the achromatic fibers entirely disappear. Then the loose chromosomes form vesicles, and occasionally these fuse together to form larger vesicles. This disturbs the nucleo-cytoplasmic relationship, and an attempt to counter balance is made by the cytoplasm breaking up and surrounding the nuclei, the larger amount of cytoplasm enclosing the larger nuclear vesicles. In some cases the cytoplasmic fragments have no nucleus whatever. These fragments with or without nuclei are later absorbed, probably by the action of phagocytic cells of follicular origin.

Newman ('13) found that in the armadillo the first maturation spindle was formed in the ovary and that the egg then waited for ovulation. Eggs not located near the periphery of the ovary were not ovulated. In such cases Newman found that only a small percentage cut off the first polar body and that only three per cent. in several hundred cases gave off the second polar body, while ninety per cent. of the eggs were struck by a process of follicular atresia, which is either a cytolysis or a parthenogenetic development. He considers only the latter contingency. In such eggs there is first a casting out of the deutoplasmic mass from the formative cytoplasm. This he considers to be an act of rejuvenation on the part of the dying cell. Released from the burden of the yolk the cell is better able to carry on the natural processes. The abstrictions of the yolk often look like multicellular structures, but Newman thinks this appearance is due to fixation. Some of these yolk fragments which he calls cytoids contain deutoplasmic granules.

Newman finds numerous tri- and multipolar spindles. These, he thinks, would result in nests of nuclei without division of the cytoplasm. Sometimes instead of a single resting nucleus formed from the maturation spindle, two nuclei might be formed instead, without the extrusion of the polar body, and suggests that from these double nuclei, the multipolar spindles might be formed. As the number of chromosomes is more than the haploid number such spindles cannot be maturation spindles. In those cells with a true cleavage spindle, Newman is inclined to the view that no polar bodies have been given off. The process is in his opinion a true parthenogenesis, but development does not proceed beyond the eight cell stage and even at that time advancing degenerative processes are to be seen.

Heape ('05) found that in the domestic rabbit copulation acted as a stimulus to certain internal rearrangements which ended in severing the ovum from its source of nourishment. At about nine hours after copulation the two polar bodies are formed and ovulation takes place about an hour later. He says: "Once freed from the ovary the mature ovum is incapable of assimilating nutriment unless it be fertilized; if from any cause fertilization is not effected, the ovum quickly dies, although it is bathed in the nutrient material supplied by the maternal tissues; ova thus degenerating are from time to time to be seen in the Fallopian tubes." It is therefore necessary for spermatozoa to be at the top of the Fallopian tube because, unlike the condition in the mouse, the ovum is dehisced from the ovary without any discus cells to provide it with food.

Bonnet ('00) reviews the different theories of degeneration and takes the view that the various spindles figured by the exponents of the parthenogenetic idea are to be considered more or less abnormal maturation spindles, and not cleavage spindles.

Van der Stricht ('01), in his study of follicular atresia in the bat, comes to the conclusion that the oöcyte of the second order forms a cleavage spindle and divides parthenogenetically. He has traced an apparent normal cleavage as far as the six-cell stage. He also described multipolar spindles and occasionally two spindles in the same egg. The first polar body is occasionally seen to divide.

Kirkham ('07) gives an excellent review of the literature on the early development of mammalian eggs previous to 1907. The common occurrence of spindles in the polar body led him to conclude that the polar body would divide mitotically. Abnormal eggs containing tripolar spindles and in one case an egg containing two spindles were observed. Kirkham agrees with Sobotta ('95) and Rubaschkin ('06) that an egg never develops after the formation of the first polar body and the second polar spindle unless fertilization takes place, the egg degenerating within the ovary or in the Fallopian tube.

Athias ('09) also takes the view that the process is entirely one of degenerative fragmentation.

Material and Methods.

The mouse is an animal well adapted to such a study, for we know from the investigations of Sobotta, Kirkham, and later of Mark and Long, that ovulation takes place without the necessity of copulation in from thirteen and three-quarter to twenty-eight and one-half hours (Mark and Long) after parturition. The sexes were kept together in suitable cages and the females examined frequently for signs of pregnancy. Whenever a female was found to be pregnant she was placed in a cage and examined each morning. As soon as a litter was found it was removed, and a record of the time made. It is from this time record that all the ages for the various eggs are figured.

Animals were killed at intervals varying from one and one half days to four days and nine hours after the finding of the litter. The body was immediately cut open and the ovaries with tube and uterus removed and placed in the fixing solution. For this purpose Zenker with acetic, and Carnoy's 6–3–1 solution, were found to be about equally good. For tube eggs strong Flemming proved excellent. The prepared sections were stained in Heidenhain's iron hæmatoxylin, Ehrlich's hæmatoxylin, or Flemming's Triple stain, usually with suitable counter stain.

Observations.

Thirty-six hours after finding the litter, eggs are almost certain to be found in the upper part of the Fallopian tube. They always show the second maturation spindle and may or may not have the polar body attached. If present, the polar body has the chromosomes arranged in the form of a spindle (Fig. 1.) During the next twelve hours there is a change in the polar bodies. The chromatin of the spindle breaks down and arranges itself into one, two or many resting nuclei. The formation of two nuclei is well illustrated in Fig. 2. Here the vesicles have been formed but a number of chromosomes or chromatin bodies have not yet entirely lost their individuality. To one side of the polar body a small body, the first indication of a mitochondrial substance, may be seen. Fig. 3 shows a later stage in which the chromatin of the polar body has formed eight vesicle-like nuclei. Since eggs with three or more polar bodies are not infrequent (see Fig. 4), one is led to believe that the cytoplasm segments, forming itself around the various vesicles. Long and Mark ('11) find that the first polar cell often divides amitotically. They consider that this aids the degeneration and absorbtion of the polar bodies due to the increase in the exposed surfaces. Kirkham also, in work which has never been published, has observed that the first polar cell occasionally divides forming a number of fragments.

The achromatic fibers of the second maturation spindle disappear early, since in only a few cases have they been noted in eggs as old as forty-eight hours. The spindle stands out clear and distinct with the chromosomes in their natural relations but no signs of fibers can be observed.

Between forty-eight and seventy-two hours a renewal of activity begins in the unfertilized egg. Its effect is first apparent in the second maturation spindle, which may break down and form a single resting nucleus. Three eggs from a seventy-six hour mouse all have a single nucleus both in the eggs and in their polar bodies. More often however numerous vesicles instead of one result from the breaking down of the spindle. An examination of Fig. 5 will make it clear how this takes place. This egg is from a mouse in which one of the uterine horns was closed by a tumor, thus preventing access of the sperm to the eggs on that side. The mouse was killed sixty hours after finding the litter. In the tube on the occluded side, two eggs were found, one in the second maturation spindle stage, and the other showing the breaking down of the spindle. In the other horn fertilization had taken place and the eggs were in the four-cell stage. In the figure it will be noted that the chromatin of the spindle has for the most part gone into the formation of a circular vesicle, in which a number of chromosomes remain practically unaltered.

A later stage picturing the breaking down of the spindle is that of Fig. 6. Here the lobes are larger and have become separated from the central vesicle. All the chromatin is now found in the walls of the various vesicles, none remaining as chromosomes.

The loss of the achromatic fibers and the breaking down of the spindle is probably due, as suggested by Kingery ('13), to the degenerative changes appearing quite early in the cytoplasm. This is shown by the fact that the cytoplasm stains more deeply than in normal eggs. Numerous dark staining granules are seen, particularly near the periphery of the egg. These constitute the mitochondrial bodies.

Kingery ('13) is of the opinion that the spindle fibers of the second maturation spindle break and free the chromosomes, which then migrate into the cytoplasm and form numerous nuclear vesicles. In my material the achromatic fibers disappear early, and from Figs. 5 and 6 it would appear that the vesicles may be formed *in situ*, and not necessarily from chromosomes which have wandered from the place of the original spindle.

In Figs. 7, 8 and 9 a different process of spindle disintegration is seen. These three eggs are all from the same mouse and were located close together in the lowest portion of the tube very near the uterus. From Fig. 7, it is evident that while the spindle fibers have disappeared; the chromosomes, although contracted and hugged closely together, still retain the form of a second spindle. Several chromatic fragments have evidently broken off and are seen scattered in the cytoplasm. The succeeding stage may be noted in Fig. 8. The individual chromosomes have separated from their spindle position and lie scattered in the cytoplasm. One of the chromosomes shows the beginning of vesicle formation, which is seen completed in Fig. 9. Fig. 11 shows essentially the same condition. The polar body has divided and several small phagocytes may be seen just inside the zona. In Fig. 10 the chromatin is arranged around the periphery of the vesicle and not clumped to one side as in Fig. 9. One notices quite a similarity between these vesicles and those in process of formation in Fig. 6, and suggests that they may have been preceeded by such a stage.

After the formation of these numerous nuclear vesicles, the fragmentation of the cytoplasm takes place. Figs. 16 and 17, adjacent sections of a seventy-two hour egg, indicate that the fragmentation may take the form of a protoplasmic budding. The large cell shown in the figure has constricted off two fragments and is forming others. Adjoining sections show five additional fragments, making seven in all. Nuclear material is present in only two of the seven fragments. Of course one cannot say that all such fragments are constricted off from the egg, for there is always the possibility that some of them may have come from the polar bodies. It has been shown by Hartman ('16) in the opossum, and by Newman for the armadillo that normally one of the first activities of the unfertilized egg is the unburdening itself of the deutoplasmic material. This is done without assistance of any spindle or apparent nuclear influence. While it cannot be said that the fragments budded off in the mouse are exclusively of yolk material, the process is essentially a similar one.

Figs. 13, 14 and 15 are later stages in the fragmentation process. They represent what Kingery ('13) calls the "morula" stage. Figs. 14 and 15 represent two adjoining sections of the same "morula," which consists of about twenty-one cytoplasmic fragments.

We have seen how the second maturation spindle breaks down and forms either a single resting nucleus or numerous nuclei, and have traced the latter to the "morula" stage. When, on the breaking down of the spindle the egg is able to form a single or only a few resting nuclei, it would seem to indicate exceptional vitality and a close approximation of the normal condition. In such an egg the process of atresia would naturally be somewhat delayed. The egg with but one or two nuclei does not fragment immediately, and it is possible that such eggs may pass from the body, as suggested by Hartman ('16) for the unfertilized egg of the opossum. This possibility is well illustrated in Fig. 18, which shows an unfertilized egg one hundred and five hours after finding the litter. Only two nuclei are present. The mitochondrial bodies (*b*) can be distinguished from the nuclei by their peculiar crystalline appearance and elliptical shape. Although these bodies are found in most of the fragmented segments to be described later, they are smaller and stain less intensely in hæmatoxylin. They are surrounded by a light area which gives them the appearance of having shrunken from the cytoplasm. In Fig. 19 the mitochondrial bodies are seen to be smaller but more numerous. In the two upper fragments they did not cut cleanly but were pushed aside by the knife. This would seem to indicate that they were much more compact and dense than the surrounding cytoplasm.

Hartman ('16) finds a similar structure in the egg of the opossum, and describes it as a homogeneous, non-granular body staining pink or lavender in iron hæmatoxylin, and bordered by a light-colored band.

The multinucleated eggs soon break up into a number of fragments, often forming two or more parts very nearly of the same size and often appearing like normal cleavage stages, were it not for the presence of extra nuclei. Figs. 12, 13 and 14 show different stages in this process.

The ultimate fate of the fragmented egg will now be considered. Very early in the degenerative process, phagocytic cells are found inside the zona pellucida, between the cytoplasm and the zona. It is impossible to mistake these cells for fragments of either the egg or polar bodies for they appear almost like transparent vesicles with the deep staining nuclear material either scattered as granules or irregularly clumped in a way which immediately suggests their origin as polymorphonuclear leucocytes. Kingery described similar cells but thought them of follicular origin. The number of these phagocytic cells inside the zona varies, eight or ten being the largest number found. These cells probably aid in the disintegration of the periphery of the cell. This process may begin some time before fragmentation sets in, as is evidenced by a study of Fig. 9. Here the space at one end of the egg filled with dark granules indicates that the cytolysis of the cytoplasm has begun.

The zona persists in nearly all cases as far as the eggs have been followed. Fig. 6 shows one of the exceptions, for here the zona is gone and many dark staining fragments which may have come either from the polar body (which is now very small) or from the broken down zona, are seen surrounding the egg. Several phagocytic cells, one of which is figured, are to be seen among the fragments. Although the zona is present in almost all cases, a careful examination usually brings to light several breaks, and it is probably through these that the phagocytic cells are able to reach the egg.

Figs. 19 and 20 show the last stages in the disintegration and absorbtion of the fragments by the phagocytes. Some of the fragments appear as mere shells with only occasional granules, while the outer edge of others shows a honeycombed condition due to excessive vacuolization.

It is found that the unfertilized egg completes its passage through the Fallopian tube and enters the uterus about the end of the third day or almost at exactly the same time as given by Sobotta for the normal segmenting egg.

Smith ('17), basing ovulation as occurring twenty hours after parturition, finds that unfertilized eggs of the mouse enter the uterus about 76 hours after ovulation and are found in the last fold of the uterus at 88 hours.

The final dissolution of the fragmented egg evidently is completed early on the fourth day, since out of eleven mice older than four days, all showing corpora lutea in the ovaries, indicating that ovulation had taken place, in only three could eggs be found. A single egg was found in the uterus of a mouse one hundred and five hours after parturition (Fig. 18).

In not a single case has anything approaching a cleavage spindle as described in atresic ova by Spuler, Loeb or Newman, been observed, but it is not to be interpreted that they may not occur, because cleavage spindles are so short lived that the study of a much greater number of eggs would be necessary before coming to any such conclusion.

SUMMARY.

The cytological changes which the unfertilized egg of the mouse undergoes in the Fallopian tube and uterus are closely similar to those which occur in the eggs of atresic follicles in the ovary.

The processes are considered degenerative and have only a superficial resemblance to parthenogenetic development.

The breaking down of the second maturation spindle of the unfertilized egg usually results in the formation of several or many nuclei. Rarely a single nucleus is formed, in which case the egg does not fragment rapidly and may pass from the uterus before the degenerative processes are complete.

The eggs with many nuclei divide into numerous cells, of which some are provided with degenerating nuclei. These are further disintegrated and absorbed by phagocytic cells, which make their way into the egg probably through breaks in the zona. The phagocytes from their appearance are polymorphonuclear leucocytes, and they evidently act on the cytoplasm causing a vacuolization of its outer portions and a later crumbling of its periphery; the end being a complete disintegration of the egg and its absorption by the phagocytes.

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EXPLANATION OF PLATES.

All the drawings are from sections of unfertilized eggs in the Fallopian tube or uterus. They were made with the aid of a camera lucida, and with the exception of Fig. 5 are reproduced at a magnification of 900 diameters.

ABBREVIATIONS.

a, zona. *b*, mitochondrial body. *c*, phagocytes. *d*, vacuole. *e*, degenerating protoplasm.

PLATE I.

FIG. 1. (48 hours.) Egg with the second maturation spindle. Polar body shows chromosomes arranged on the spindle fibers. Eggs of this type, showing no changes are found in the tube up to forty-eight hours after parturition.

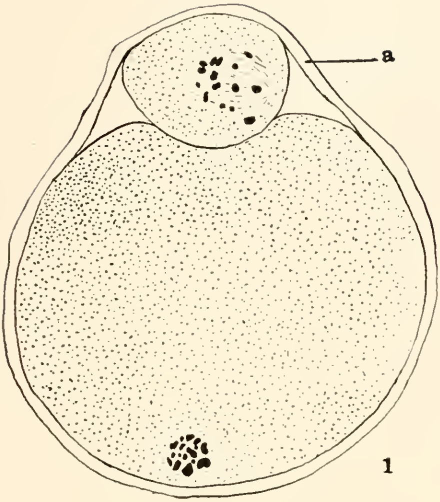
FIG. 2. (56 hours.) In this egg the spindle is present but the chromosomes seem to have clumped together more than in Fig. 1. The spindle of the polar body has disappeared and in its place are seen two resting nuclei.

FIG. 3. Polar body (60 hours) from same mouse as Fig. 5 showing many resting nuclei formed from the breaking down of the polar spindle or by the chromatin fragments often observed in the polar body.

FIG. 4. (48 hours.) Showing the fragmentation of the polar body. Here the first polar body has divided once and one of the parts has redivided. The spindle cut somewhat obliquely is one of few showing good achromatic fibers. These fibers seem to end in a centrosome-like body.

FIG. 5. (60 hours.) Chromosomes of the second maturation spindle breaking down and forming resting nuclei. Vesicular lobes are seen extending out from the main vesicle. Quite a number of the chromosomes have not yet entered into the process and are seen in the lobes of the forming nuclei. This mouse had a tumor in one of the uteri which prevented fertilization, while on the other side, eggs in the tube had reached the four cell stage. (× 1,800.)

FIG. 6. (78 hours.) A later stage of vesicle formation from the maturation spindle. Chromatin all in the vesicles, no chromosomes as such. This was one of the few eggs in which the zona was missing. Many dark staining granules surrounded the egg and in among them several phagocytes. One phagocyte shown at left of polar body, which is much smaller than normal, suggesting that the phagocytes have been acting upon it and probably upon the zona also.



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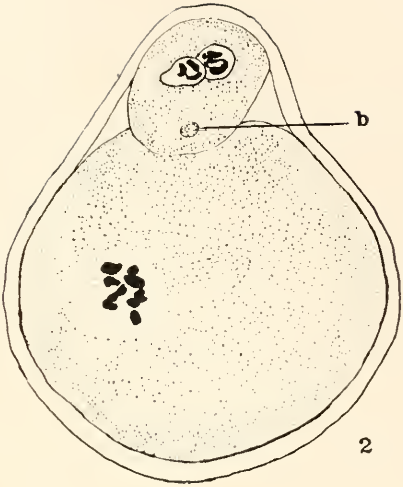

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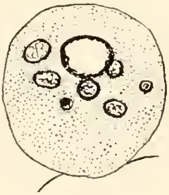

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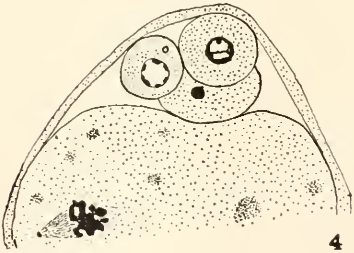

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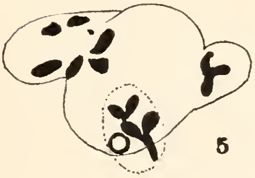

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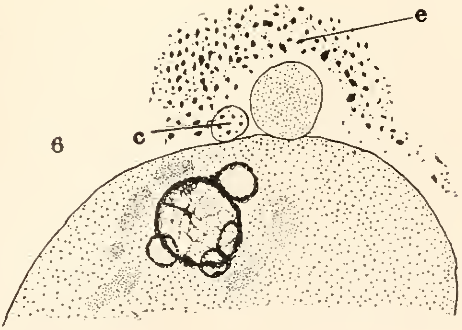

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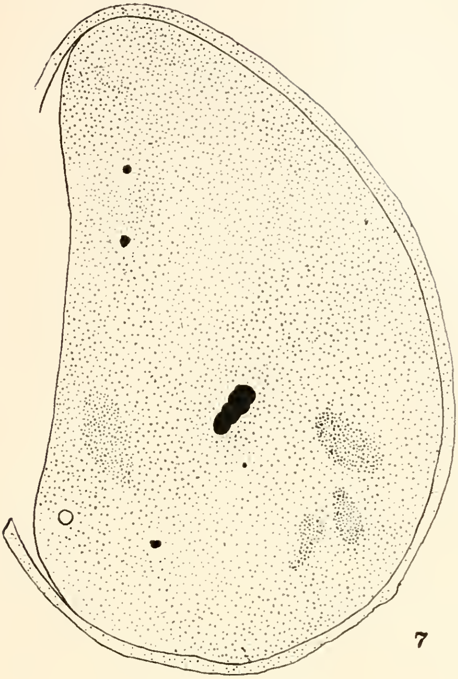
Plate II.

Fig. 7. (72 hours.) Spindle fibers missing, chromosomes of spindle hugged closely together. Several parts separated and out in the cytoplasm.

Fig. 8. (72 hours.) A later stage of the above. Chromosomes scattered in the cytoplasm. One showing the beginning of vesicle formation. Several phagocytes are present inside the zona. Cytoplasm near open end shows beginning of disintegration.

Fig. 9. (72 hours.) Completion of vesicle formation begun in Fig. 8. The chromatic material clumped to one side of the vesicle. Quite a few small particles of disintegrated cytoplasm may be seen inside the zona indicating the activity of the phagocytes.

Fig. 10. (72 hours.) Reconstruction showing ring-like formation of nuclear material. Possibly a succeeding stage to Fig. 6. A phagocyte cell is noticed just outside the zona, which is very faintly stained.



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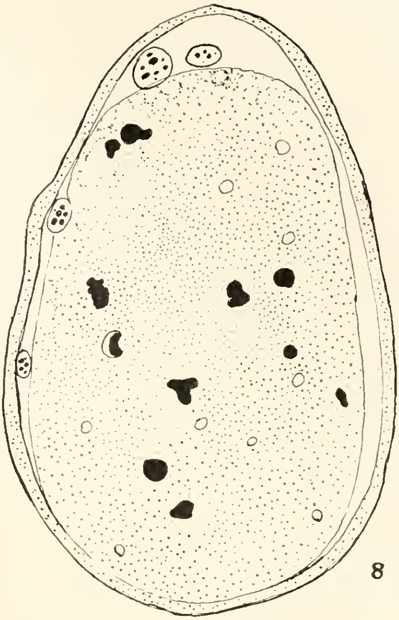

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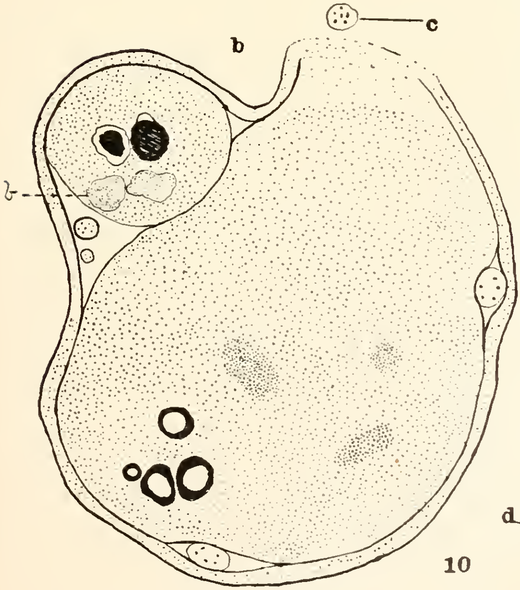

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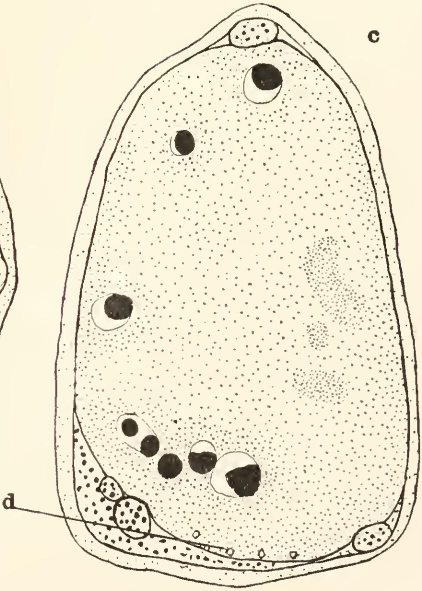

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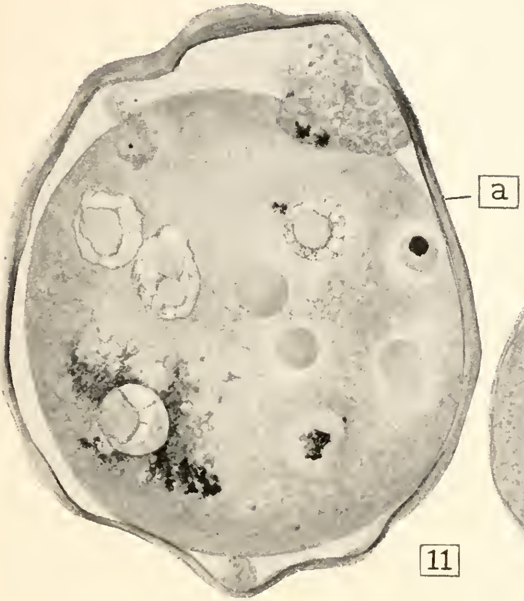
PLATE III.

FIG. 11. (72 hours.) Egg showing four resting nuclei, and a number of mitochondrial bodies. In addition a divided polar body and two or more smaller structures which are probably phagocytic cells.

FIG. 12. (72 hours.) Fragmented egg looking very much like a normal two-cell stage, except that each blastomere has two or three nuclei. The nuclei contain nucleoli and appear perfectly normal.

FIG. 13. (81 hours.) A later stage of fragmentation. A large resting nucleus is seen in one of the larger segments, while other fragments have none. In two of the cells the mitochondrial body is quite conspicuous.

FIG. 14. Egg of uncertain age, consisting of twenty-one fragments. Numerous phagocytic cells are seen in and about the fragments. Zona entirely missing.



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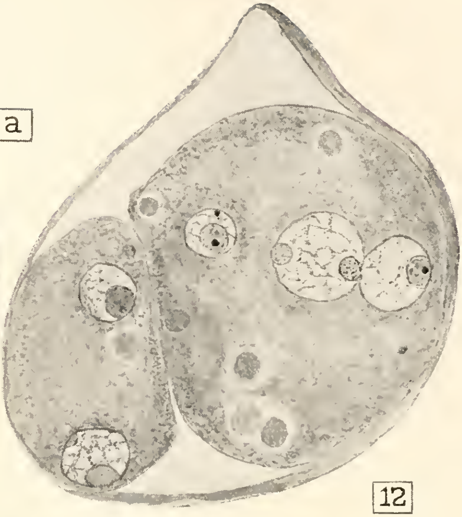

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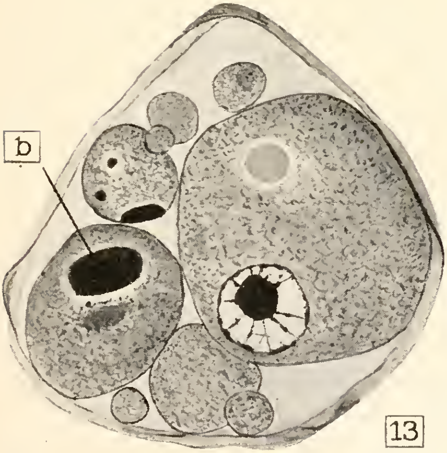

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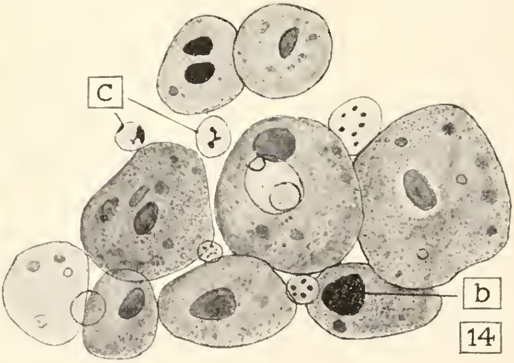

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Plate IV.

Fig. 15. Drawing of adjacent section to that shown in Fig. 14.

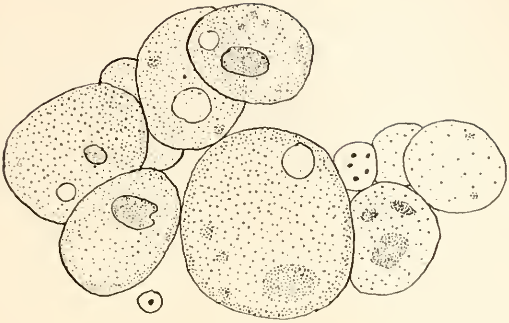
Fig. 16. (72 hours.) Egg showing the formation of protoplasmic fragments independent of any spindle. These are later pinched off and form small segments inside the zona.

Fig. 17. Drawing of adjacent section to that shown in Fig. 16. A study of all the sections of this egg shows that seven fragments have been pinched off, and that of these only two appear to contain nuclear material.

Fig. 18. (105 hours.) Egg is without zona, contains two nuclei and two immensely large refractive mitochondrial bodies. The polar body still remains attached to the egg.

Fig. 19. An 81-hour egg showing phagocytic effect. In two upper cells the mitochondrial bodies have been pulled out of the cytoplasm towards the left by the knife, leaving clear spaces where they originally were, indicating the solid character of these structures. Many vacuoles are seen around the border of the fragments. Between the cells dark stained particles of degenerated cytoplasm is being absorbed by the phagocytes.

Fig. 20. (81 hours.) A later stage than Fig. 19. In one of the fragments hardly any content is to be seen except a few scattered granules.



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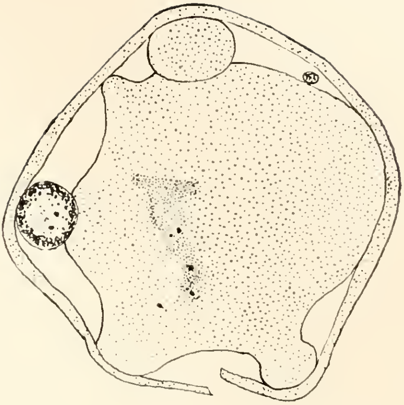

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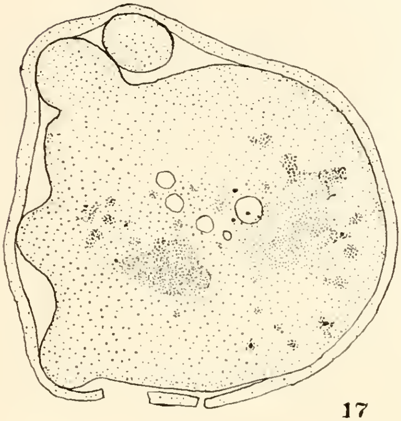

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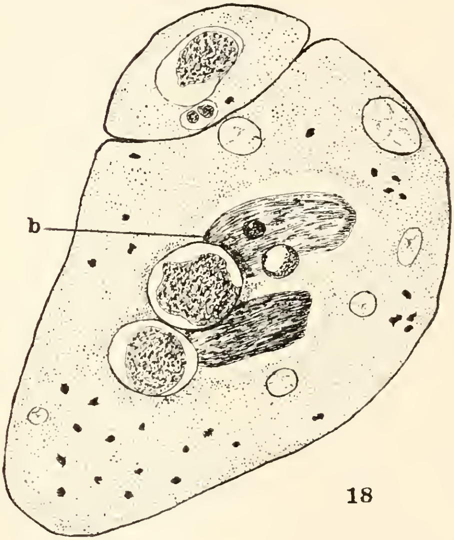

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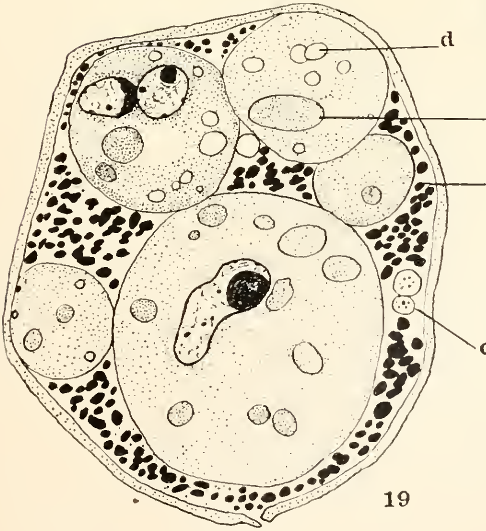

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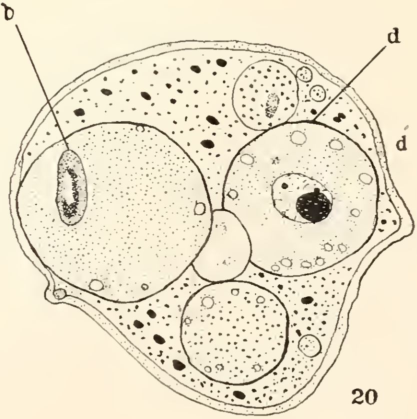

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H. W. CHARLTON.

OBSERVATIONS ON THE STRUCTURE OF DOUBLE MONSTERS IN THE EARTHWORM.[1]

ROXIE A. WEBER.

I. Introduction.

The work which has thus far been done on double monsters in the terricolous group of annelids is quite limited, since in only a few species, *Lumbricus trapezoides*, *Lumbricus terrestris*, *Allolobophora fœtida* and *Allolobophora trapezoides*, has this condition been observed. The first of these forms is described by Kleinenberg, the remaining three by Vedjovsky. These two investigators agree neither as to the method of union nor as to the cause of this phenomenon. It is not the object of this paper to give the factors influencing such monster formations, but merely to describe the manner in which the members are joined.

Material and Technique.—The material studied consists of twelve double embryos, the shortest member of which is at least 60 segments in length, the longest 125, one monster in which one individual consists of 16 the other 17 segments, 19 double gastrulæ and two embryos showing a bud on one side of the blastopore, and one case of an egg consisting of two distinct hemispheres connected by a band of large cells. The entire supply was obtained from Professor Patterson, of this University.

The fixing fluids employed were Meves, Gilson, and Bouin, all of which gave only fairly good results. Ehrlich's hæmatoxylin was used as a stain.

The species studied is *Helodrilus caliginosus trapezoides*, according to Professor F. Smith's identification.

Review of the Literature.—Before describing the individuals studied, it will perhaps be well to give a brief review of the results of the two authors above mentioned.

Kleinenberg found that the number of eggs in the capsules of *L. trapezoides* varies from three to eight of which usually one, though sometimes two or three, produces an embryo. The remaining ones, not becoming fertilized, disintegrate completely.

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[1] Contributions from the Zoölogical Laboratory of The University of Texas, No. 139.

By a series of somewhat irregular cleavages the egg develops into a germinal bladder, one layer in thickness (Pl. IX., Fig. 2, Kleinenberg). Thereupon a two-layered condition begins to appear, one side of the cell mass becoming differentiated in advance of the other. At this pole all the cells divide rapidly except two, which become pushed in and covered over by the small blastomeres. From these two cells (mesoblasts) are derived the first rudiments of entoderm and mesoderm (Pl. IX., Fig. 4).

While this elongation is taking place a transverse furrow appears midway between the two ends extending almost entirely around, leaving the two hemispheres connected by only a few enlarged ectodermal cells.

When this stage has been reached the cells of the other pole begin to undergo the same changes and finally we have formed from each half an embryo joined in varying degrees to the other by a band of ectodermal cells. The separation is eventually effected by a series of rotations which usually result in the breaking of the uniting cord. When for one reason or another this is not accomplished we have true monstrosities in all degrees of coalescence.

According to Kleinenberg the union never extends to the internal organs but is confined to the external epithelium of the body wall.

The monsters formed in this manner are of the same or nearly the same size. There is however still another type of abnormality (Pl. IX., Fig. 10), *e. g.*, those showing bud formation. Kleinenberg explains their production as being due to a very unequal development of the two halves of the above mentioned cell mass.

In all his work he has found only a few cases in which two individuals did not emerge from one capsule and in those few exceptions rudiments of a second were usually found. For this reason he concludes that each egg produces two individuals normally.

Vedjovsky on the other hand holds this condition to be abnormal. He explains the formation of double embryos as being due to what he terms *Doppelfurchung*. The egg elongates in the direction of the animal pole and subsequently divides into two blastomeres either of equal or unequal size. They are designated as A and a. From each of these cells there is produced a quartet, one lying upon the other giving the appearance of micro- and macromeres in the cleavage of *Crepidula* or the eight cell stage of *Synapta*, depending on the proportionate sizes of A and a. Each of the quartets finally gives rise to an individual which remains attached to its companion. If the a cell is smaller than A, there is produced one well developed individual and a bud.

This author has found examples of monster formation in *Lumbricus terrestris*, *Allolobophora fœtida* and in *Allolobophora trapezoides*. In the first group he found two cases in both of which the two individuals were joined on the dorsal side through one segment. Only one monster was found among several hundred embryos of the second group. In the third group a large number was found which the author describes under three divisions.

A. Those monsters in which the individuals are united on the ventral side along the entire length of the body (Pl. 19, Fig. 14).

B. The cases in which the members are joined on the dorsal side (Plate 21, Fig. 7).

C. The double monsters in which the individuals are fused end to end. (*a*) Those in which both members are of similar length (Pl. 21, Fig. 9). (*b*) Those cases in which one member is rudimentary (Pl. 19, Fig. 12).

II. OBSERVATIONS.

Even though these two workers differ so markedly in their conclusions it will be noted from the following that the observations made by the writer are similar in many respects to those made by both of these men.

In this study of the monsters of *Helodrilus caliginosus trapezoides* it has been found that the greatest variety exists in the structure of the nervous system. There is in every case a complete union of the five layers of the body wall, and the digestive tract is either fused or not fused, but the nervous system is modified in a variety of ways. The manner and extent of union of these individuals and their organ systems are best described in the following groups.

1. Those double monsters in which the union is dorsal. This group may again be subdivided into—

(*a*) Those in which the union extends to the alimentary tracts. Of these there are two examples, Nos. 95 and 171, Plate I. From Figs. 1 and 2, Plate I., it can be seen that the fusion in No. 171 is through one segment only, while in No. 95 it extends through five. A description of sections through this monster will serve to show the general relationship of the organ systems in the members of this group. Fig. 3, Plate I., is a section through the anterior portion showing the condition of the nervous system. It will be seen that there is almost a continuous band of nerve tissue extending around the pharynx. A study of the neighboring sections shows that this band is really complete. It will also show that the portion marked (*b*) in the figure enlarges into a bilobed brain and that a similar structure exists on the opposite side of the pharynx. At the same time there is also to be found at each end of the greatly elongated pharynx another set of ganglia. It would be impossible here to determine which are brain and which are ventral cord ganglia were it not for the presence of setæ (*s*). This at once leads to the conclusion that the ganglia at the end of the pharynx must be those of the ventral cord. This inference is further proven by a study of sections through a more posterior region, as shown in Fig. 4, Plate I. Here are no longer to be found the ganglia on either side of the pharynx but only those at the opposite ends. This section too shows the setæ in normal relationship with the ventral cord and the nephridæ in their natural position. They are shown only on one side but those of the other half can be seen in the next section.

It will be noted that the single pharynx is greatly elongated from end to end of the monster. Throughout the fused part it remains as one cavity dividing into two parts only when the point of separation of the two members is reached, one passing into each individual. Surrounding the pharyngeal cavity there is a great mass of muscular tissue attached by thin strands to the body wall. This condition is exactly similar to that found in single individuals.

The sub-intestinal blood vessels are clearly visible (*v.b.v.*) in their normal position between the alimentary tract and the nerve cord. On each side of the muscular pharynx there lies a more or less regular vessel (*D.B.V.*) which when traced to the point of separation of the two members will be found to approach the center and then one passes into one individual, the other into the other, the vessel on the left side passing into the upper half, the one on the right into the lower half.

Though the other monster is formed by a coalescence of the two members through one segment only, that union is complete in every respect from body wall to pharynx. Fig. 5 is a longitudinal section through No. 171 showing a portion of one of the brain ganglia together with a small part of the nerve cord of each of the individuals. A study of the successive sections will show that the pharynx arises from a flattened portion which is lined with the same kind of epithelial cells as the rest of the digestive tract and which lies between the two members as indicated in Fig. 2, Plate I. (*m*). The ventral cords are found to be connected to the cerebral ganglia, of which there are two sets, one on each side of the mouth opening, by means of commissures as in No. 95.

The setæ, not shown in this figure, and the nephridæ lie along the same side of the body as the nerve cord.

(*b*) Those in which the alimentary tracts have not become united. Nos. 91, 92, 142 and 173 shown in Plate II as Figs. 6, 7, 8 and 9 illustrate this group. It will be noted that though only one definite pair of segments appear to be fused in the first three cases, there is in each instance an irregular mass of tissue between the separated edges of the fused pair of segments. Whether these masses are modified segments has not been determined.

In all four instances the nerve cords are formed on the side opposite the line of fusion. These cords are joined to the bilobed brains in a manner very similar to that found in No. 95 (Fig. 3). It is to be noted that the union of these individuals is the same in kind and extent as in the group already described except for the digestive tract. In every one of the four there is distinguishable a separate alimentary tract for each member beginning with the mouths. These structures lie very near the nerve cord and are bounded on the inner side by a mass of muscles similar to those which usually lie on either side of the elongated fused pharynx.

The fixation of the blood vessels was such that they could not be studied.

The members of these groups to which belong half the specimens studied, have undoubtedly been fused along the dorsal side. The relative positions of setæ, nephridæ, and nerve cord are positive proof thereof. Vedjovsky gives a number of figures, very similar to Figs. 3 and 4, in his paper but he explains the union as being along the ventral side. It is impossible to disprove this conclusion for the two members were joined along their entire lengths. In the cases described in this paper, the union was never through more than five segments If, then, the coalescence had been other than dorsal, it is very probable that the nerve cord together with the setæ and excretory organs would have swung around to their natural position in the separated portions of the two individuals. This, however, is not the case. Therefore, the conclusion must be drawn that at least in these instances the union is no other than dorsal.

It is very probable that the one case described by Vedjovsky is also one of dorsal instead of ventral union, and that the cerebral ganglia are composed of half from each member instead of this being the case for the ventral cords, as that author supposes. If those individuals in which the digestive tract was separate had not been found it would not be possible to make this statement for it might be argued that each side of the pharynx was formed from the alimentary tract of one member. But since those cases have been found it can be easily seen that a joining of these structures would have resulted in forming a greatly elongated organ similar to that shown in Vedjovsky's figures. Furthermore, if the union in these forms had been ventral, the pharynges would certainly not have been found in the positions in which they lie, but would have been much nearer together in the central portion and side by side instead of end to end.

2. Those cases in which the union is latero-dorsal. Thus far only one example has been found of this group, individual No. 70 shown in Fig. 10, Plate II. Figs. 11 and 12, Plate II., show sections through the joined region which in this case extends through three segments. The first is very much more anterior than the second as is indicated by the presence of the cerebral ganglia (*bg*) of which there is in this monster only one set. The commissures extending from it can be seen in the same figure in which is also present the beginning of one of the nerve cords (*nc*). A study of the next few sections shows the connection of the brain to the two cords and also a connecting band between these two structures. In this manner the single greatly elongated alimentary tract, which in this case is the result of a union, is completely surrounded by nerve tissue. Fig. 12 gives a section through a more posterior region showing the relative positions of the ventral cords.

In no part of the joined portion can there be found less than eight pairs of setæ indicating that the union does not extend beyond the sides of the individuals.

The nephridæ also are in their normal number and relationship to the ventral cord.

3. This is the group in which the union is end to end and in which the cerebral ganglia are to be found on the opposite side of the digestive tract from the ventral cord. It can be seen from an inspection of Figs. 13, 14 and 15, external drawings of Nos. 90, 93 and 2, that the extent of union is limited to an unusually small area. Sections show that the union is across the dorsal side, for the ventral cords lie on the sides opposite that area, while the cerebral ganglia lie on the same side.

The digestive tracts have a common origin in each of the three monsters arising from a single mouth opening from which the pharynx passes into each individual. There is no noticeable difference in the structure of any of the fused organs of these forms as compared with those of No. 95 except in the nervous system.

A study of No. 90 will show a condition of that system somewhat different from any so far described. The ventral cord of each individual lies on the side opposite the union while the brains lie on the same side and are connected to the cords by means of commissures, a condition very similar to that found in normal single individuals. The brains however in this instance are joined to each other. In one of the individuals the commissural connection between brain and cord can be seen beyond the point of separation, but in the other member the two ganglia of the brain seem to be separated and joined separately to the cerebral ganglia of the first member.

In No. 93, shown in Fig. 14, there is present only one set of cerebral ganglia greatly elongated. It lies on that side of the pharynx opposite the cords and is joined to that structure in one member by two commissures, to that in the other by only one.

A study of sections through No. 2 reveals a condition similar to that in No. 90. There are two distinct sets of cerebral ganglia, one joined to each nerve cord as in normal individuals and a connection between the two brains much as between the two cords in No. 70.

4. That group of double monsters in which the union between the two individuals has been side to side with both mouth openings on the same side, illustrated by Nos. 67 and 1.

In No. 1 the union is through one segment only as indicated in Fig. 16, Plate III. There is a single mouth opening lined with the columnar epithelial cells, from which the digestive tract of each individual passes inward. There can be seen on one side of the pharynx in a transverse section a single set of cerebral ganglia which is joined to the ventral cords by commissural strands extending over the pharynx. There are no such structures to be found on the under side of the alimentary tract. On the opposite side of the section there can be traced a connection between the cords themselves. Fig. 17, Plate III., is a section through the united portion of No. 1 showing the single pharynx, cerebral and ventral ganglia and a portion of the strand of nerve tissue connecting the two cords.

A study of sections through No. 67, shown in Fig. 18, Plate III., will show that the two mouth openings have been joined into one, and that the ventral cords lie on one side of the common pharynx and the cerebral ganglia on the other. There is only one set of brain ganglia to be found in this monster, but, unlike all other cases, there are three distinct commissural strands joining it to the nerve cords, one extending over the top of the pharynx, the other two below the point of union of the two digestive tracts forming a triangle with each other. Fig. 19, Plate III., is a cross section of these last mentioned strands and their connection to the two cords. As in No. 70 there is in this monster also a limited connection between the two cords.

5. Cases in which the two individuals are extremely unequal in size, *e. g.*, bud formation. Thus far there have been found two such cases, a section of one being shown in Fig. 20, Plate III.

III. Discussion.

It will be remembered that reference was made to the difference in view concerning the origin of double monsters in an earlier part of this paper. Vedjovsky maintains that the formation of two embryos from one egg is abnormal, whereas Kleinenberg maintains the opposite. A difference of this kind may be explained on the basis of the difference in the forms worked on by these two investigators. This paper is a study of a form very similar, in its mode of development, to that described by Kleinenberg. It has been found that it is quite common, not only for monsters to appear in this group, but also for two individuals to emerge from one capsule. Out of the 184 cocoons opened 57 contained only one individual, 101 two, and 25 eggs in various cleavage stages and 1 four embryos. Thirty-five of the 101 cases were in the form of monsters. Six cases have been noted in which two or more eggs were present but in four of these only one was undergoing development while the others were in various stages of degeneration. One egg was found which had just reached a stage in development sufficiently far advanced to show the division into two hemispheres and the connecting band of larger cells very similar to Kleinenberg's Fig. 6. Three others were found which had begun to elongate in a manner very similar to the above.

It would certainly be safe to conclude that it is a common occurrence for one egg of this species to give rise to two individuals. If this were not the case it would seem rather unusual to find so large a number of cases in which two and only two individuals emerge from one capsule when the number of eggs found in one capsule varies ordinarily from three to eight. The elongation of those eggs sufficiently far advanced in development to show this phenomenon is still further indication of this same thing.

Kleinenberg attributes the cause of this double embryo formation to the entrance of two sperm into a single egg setting up two points of activity. In the light of more modern discoveries this explanation no longer holds good. Vedjovsky suggests the possibility of temperature and moisture changes, and exposure to air bearing their influence on the egg and causing its abnormal development. Until further studies are made along this line it will be impossible to state any definite causes of monster formation.

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Explanation of Plate I.

Helodrilus caliginosus trapezoides.

1. No. 95, showing union through five segments.

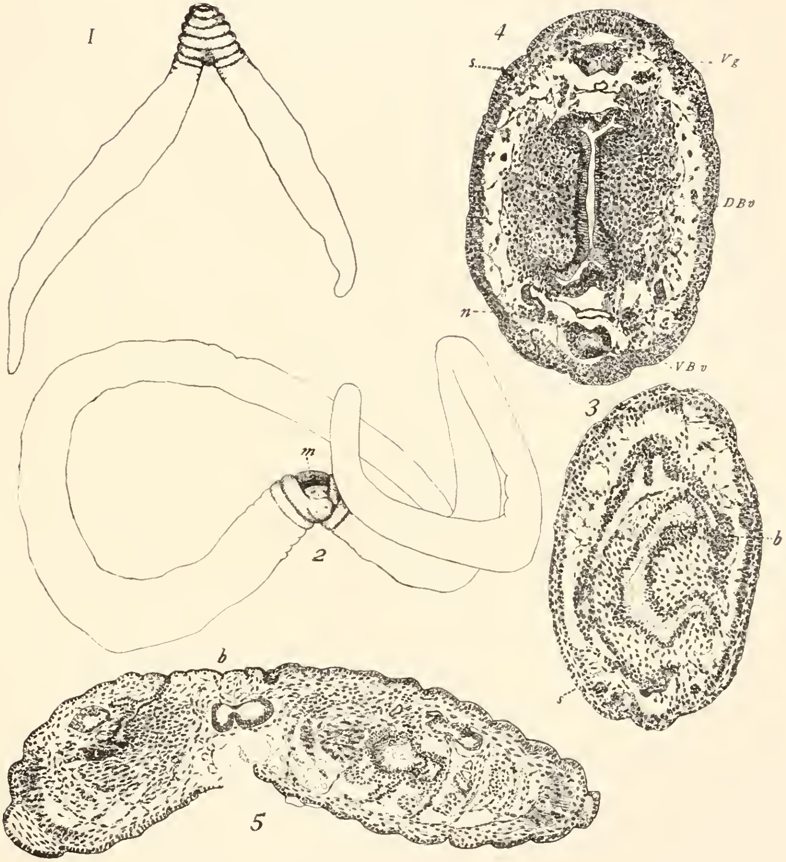
2. No. 171, showing union through one segment.

3. Section through the anterior portion of No. 95, showing the pharynx fused and the extension of the nerve tissue almost entirely around the pharynx. *b*, cerebral ganglia; *s*, setæ.

4. A section through a more posterior region of the same monster. *Vg*, ventral ganglia; *DBv*, dorsal blood vessel; *VBv*, ventral blood vessel; *n*, nephridium; *s*, seta.

5. A longitudinal section through No. 171, showing a portion of the cerebral ganglia *b*, and also parts of the ventral cords.

PLATE I.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Vg
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ROXIE A. WEBER.

EXPLANATION OF PLATE II.

Helodrilus caliginosus trapezoides.

6. No. 91.
7. No. 92.
8. No. 172.

Fused through one segment only with irregular masses between the separate edges of the united segment.

9. No. 173, united through four segments.

10. No. 70, united through four segments.

11. A section through the anterior portion of No. 70, showing a single set of cerebral ganglia (*bg*) and one of the ventral ganglia (*nc*).

12. A more posterior section of the same monster showing two ventral ganglia.

13*a* and *b*. No. 90.
14. No. 93.
15. No. 2.

All show very limited connection.

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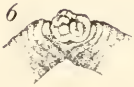

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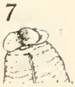

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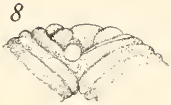

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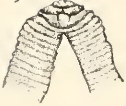

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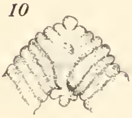

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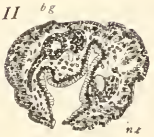

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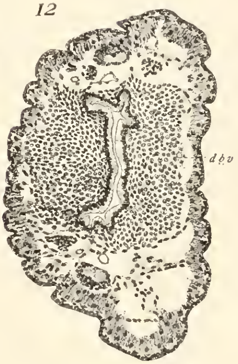

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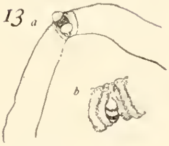

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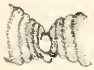

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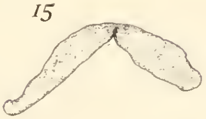


ROXIE A. WEBER.

EXPLANATION OF PLATE III.

Helodrilus caliginosus trapezoides.

16. No. 1 viewed from the side of the mouth opening.

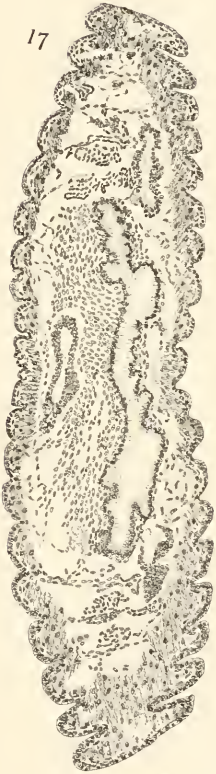
17. A section through No. 1 horizontal to the mouth opening, showing the cerebral ganglia on one side of the pharynx and the cord tissue on the opposite side.

18. No. 67, united through two segments.

19. A transverse section through the posterior part of the united portion showing the cerebral ganglia joined to the ventral ganglia (*vg*) by commissures.

20. A section through a gastrula stage showing bud formation.

17



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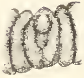

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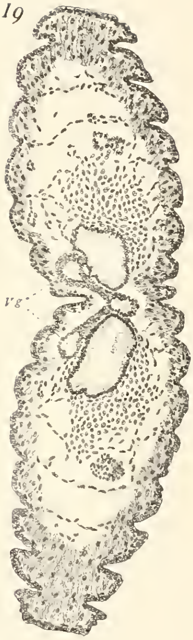

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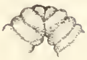

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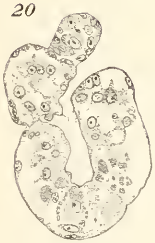

ROXIE A. WEBER.

IODINE AS A PARTHENOGENETIC AGENT.

ALVALYN E. WOODWARD AND FLORENCE S. HAGUE.

In the course of some experiments with *Arbacia* eggs at Woods Hole last summer, it was noted that, if a little iodine was added to the eggs in sea-water, regular fertilization membranes appeared on a large number. This led to an attempt to find the optimum conditions for the use of this reagent in parthenogenesis.

The stock solution used was made by dissolving iodine crystals in sea-water to the point of saturation. This stock solution was diluted with sea-water as shown in the tables.

Some of the females were stimulated to shed their eggs into watch glasses. In other cases, the ovaries were removed and shaken in sea-water to loosen them. Whichever method was followed, the eggs were washed and allowed to settle. The supernatant water was poured off, the eggs evenly mixed in fresh sea-water, and 2 c.c. of the mixture put into each of a series of watch glasses. To each of these an equal volume of iodine solution was added, for a given length of time. Some of the eggs were then put into finger-bowls containing about 50 c.c. of sea-water. Others were treated for 20 minutes with hypertonic sea-water (50 c.c. sea-water + 8 c.c. 2.5 N NaCl). At the end of that time, the solution was drawn off and the eggs transferred to finger bowls of sea-water. With each lot of eggs, three controls were made, one fertilized with sperm, one uninseminated, and one uninseminated but treated with hypertonic sea-water.

Table I. shows the percentage of membranes and cleavages produced by varying the strength of the solution and the length of exposure. To obtain these averages, over two hundred eggs were counted in each case.

It will be noted that there is great variability in the results with different batches of eggs. This may be explainable from the fact that the experiments were carried on during the last two weeks of August and the first week of September, a period

TABLE I.

PERCENTAGE OF CLEAVAGES AND MEMBRANES OBTAINED BY TREATING ARBACIA EGGS WITH IODINE, OR IODINE FOLLOWED BY HYPERTONIC SEA-WATER.

Time, Min.	Sat. Iodine Solution.		$\frac{1}{2}$ Sat. Iodine Solution.		$\frac{1}{4}$ Sat. Iodine Solution.		$\frac{1}{8}$ Sat. Iodine Solution.		$\frac{1}{16}$ Sat. Iodine Solution.		$\frac{1}{32}$ Sat. Iodine Solution.		$\frac{1}{64}$ Sat. Iodine Solution.		Controls.			Remarks.
		+ Hyper-tonic.		+ Hyper-tonic.		+ Hyper-tonic.		+ Hyper-tonic.		+ Hyper-tonic.		+ Hyper-tonic.		+ Hyper-tonic.	Fertil-ized.	Not Fertilized.		
																In Sea-water.	In Hyper-tonic.	
2.5			14	24											69	3	5	Shed
			14	22											64	2	2	Shaken
	2	7	17	16											68	4	2	Shaken
					26	27	16	16							41	1	4	
					16	32	11	39							69	3	11	
									12	13	12	14			85	7	9	
									5	8	4	12			83	7	9	
5					16	12	14	39	6	16	3	3			44	2	2	Shed
					18	21	30	22	17	17	5	15			32	1	2	Shed
					3	15	20	22	20	23	9	10			83	1	4	Shaken
			1	1	21	2	20	29	14	23					82	5	6	Both
							12	32							3	1	1	Shed
							19	20							94	5	6	Shaken
							16	27							94	5	6	Shaken
									8	21					66	3	5	Both
			20	17											69	3	5	Shed
			19	7											64	2	2	Shaken
			18	9											68	4	2	Shaken
	1	1			49	49	11	5							41	1	4	
					23	37	26	43							69	3	11	
									10	13	9	4			85	7	9	
									2	13	5	10			83	7	9	

TABLE I.—*Continued.*

Time, Min.	Sat. Iodine Solution.		$\frac{1}{2}$ Sat. Iodine Solution.		$\frac{1}{4}$ Sat. Iodine Solution.		$\frac{1}{8}$ Sat. Iodine Solution.		$\frac{1}{16}$ Sat. Iodine Solution.		$\frac{1}{32}$ Sat. Iodine Solution.		$\frac{1}{64}$ Sat. Iodine Solution.		Controls.			Remarks.
		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.	Fertilized.	Not Fertilized. In Sea-water.	Not Fertilized. In Hypertonic.	
7.5			5	21	29	20	16	13	1	2					13	0.	1	Shed
			35	37	28	28	41	31	8	18					70	4	10	Shaken
							21	47							3	1	1	Shed
							30	22							94	5	6	Shaken
							22	27							94	5	6	Shaken
									9	26					66	3	5	Shed
			15	13											69	3	5	Shed
			11	10											64	2	2	Shaken
	7	4	23	16											68	4	2	Shaken
					34	24	24	27							41	1	4	
					25	36	28	27							69	3	11	
10			13	7	23	20	21	19	11	16					88	1	5	Shed
			0	15	30	26	25	19	8	21					51	0	3	Shed
			3	9	37	25	13	33	7	17					88	1	11	Shaken
			3	15	22	16	14	18	11	6					13	0	1	Shed
			28	23	19	23	11	21	23	32					70	4	10	Shaken
			1	1	1	8	26	31	28	28					82	5	6	Both
							45	56							3	1	1	Shed
							19	24							94	5	6	Shaken
							26	37							94	5	6	Shaken
									16	24					66	3	5	Both
			8	5											69	3	5	Shed
			7	14											64	2	2	Shaken
	3	3	11	17											68	4	2	Shaken

TABLE I.—*Continued.*

Time, Min	Sat. Iodine Solution.		½ Sat. Iodine Solution.		¼ Sat. Iodine Solution.		⅛ Sat. Iodine Solution.		$\frac{1}{16}$ Sat. Iodine Solution.		$\frac{1}{32}$ Sat. Iodine Solution.		$\frac{1}{64}$ Sat. Iodine Solution.		Controls.			Remarks.
		+ Hyper-tonic.		+ Hyper-tonic.		+ Hyper-tonic.		+ Hyper-tonic.		+ Hyper-tonic.		+ Hyper-tonic.		+ Hyper-tonic.	Fertil-ized.	Not Fertilized. In Sea-water.	Not Fertilized. In Hyper-tonic.	
10					32	44	18	14							41	1	4	
					26	39	18	44							69	3	11	
									15	12	4	15			85	7	9	
									6	17	4	10			83	7	9	
12.5							8	28							3	1	1	Shed
							13	18							94	5	6	Shaken
							23	24							94	5	6	Shaken
									16	26					66	3	5	Both
			12	6											69	3	5	Shed
			7	19											64	2	2	Shaken
	11	3	8	16											68	4	2	Shaken
					18	20	19	46							69	3	11	
15							15	33							3	1	1	Shed
							16	22							94	5	6	Shaken
							17	41							94	5	6	Shaken
									21	35					66	3	5	Both
					24	42	29	29							69	3	11	
									9	11	10	12			85	7	9	
					21	27	7	13					2	5	92	1	2	Shaken
20									15	17	14	15			85	7	9	
					21	24	9	5					2	8	92	1	2	Shaken
25									15	20	8	16			85	7	9	

near the close of the breeding season, when the eggs vary greatly in their fertilizing power and their sensitiveness to reagents. It is probable that the strength found best for this season will prove too great for the more sensitive eggs in the height of the season—July, for instance.

Table II. is a summary of Table I., giving the average number

TABLE II.

SUMMARY OF TABLE I.

Time, Min.	Sat. Iodine Solution.		$\frac{1}{2}$ Sat. Iodine Solution.		$\frac{1}{4}$ Sat. Iodine Solution		$\frac{1}{8}$ Sat. Iodine Solution.		$\frac{1}{16}$ Sat. Iodine Solution.		$\frac{1}{32}$ Sat. Iodine Solution.		$\frac{1}{64}$ Sat. Iodine Solution.	
		+ Hyp.		+ Hyp.		+ Hyp.		+ Hyp.		+ Hyp.		+ Hyp.		+ Hyp.
2.5	*2*	*7*	15	21	21	30	14	28	9	11	8	13		
5	*1*	*1*	15	9	22	23	19	27	11	18	6	8		
7.5	*7*	*4*	18	19	29	27	26	28	6	15				
10	*3*	*3*	6	12	24	25	20	26	14	19	4	13		
12.5	*11*	*3*	9	14	*18*	*20*	16	29	*16*	*26*				
15					23	35	17	28	15	23	*10*	*12*	*2*	*5*
20					*21*	*24*	*9*	*5*	*15*	*17*	*14*	*15*	*2*	*8*
25									*15*	*20*	*8*	*16*		

of cleavages plus membranes obtained by each method of treatment. A number in italics is the result of one experiment, rather than the average of several. There is a remarkable uniformity in the percentages obtained by treating the eggs for varying periods of time. For instance, whether exposed for two and one half or fifteen minutes, or for some intermediate period, to one eighth saturated iodine, the eggs gave percentages between 26 and 28. The indication is that the iodine enters the egg immediately and affects it immediately to its full extent. One would expect this result if it acted by combining chemically with something in the egg. While length of exposure has no effect, varying the strength of iodine used causes great variation in the result. This indicates a secondary, non-initiatory or injurious effect due to the excess. The optimum results usually followed treatment of 2 c.c. eggs in sea-water with 2 c.c. of one fourth or one eighth saturated iodine solution. While hypertonic after treatment was nearly always beneficial, in most cases the effect was so slight as to be negligible.

It may be recalled that membrane formation and subsequent cytolysis were obtained by Loeb,[1] McClendon,[2] and Glaser,[3] in *Arbacia* eggs, by placing them in sea-water diluted with distilled, or in pure distilled water. In the latter case, the eggs became "ghosts" of their former selves, since the pigment dissolved out and increased osmotic pressure caused the egg to absorb water and swell enormously. If an excess of iodine is added to normal eggs, they also become pale, but due to a precipitation of the pigment, which gathers as a small dark mass near one side of the egg. There is seldom indication of swelling or ghost formation.

The membranes obtained by the iodine method were identical with the true fertilization membranes which appear when the sperm enters the egg. They do not resemble the membranes formed by treatment with butyric acid and hypertonic sea-water, which Loeb describes as very difficult to see, excepting by the trained observer. This normal appearance of the membranes was noted by others accustomed to distinguishing types of membrane in *Arbacia*. The significance of the nature of this membrane will be discussed in a later paper

Reference to Table I. brings out a result that was wholly unexpected. It is commonly supposed that eggs shed by the animal are in better condition and more fertile than those taken from the ovary. The results with one female, whose shed eggs produced only 3 per cent. cleavages, while those shaken from the same ovaries gave 94 per cent., were so striking that the averages were computed. It was found that the shed eggs averaged only 43 per cent. as against 80 per cent. cleavages from the eggs shaken from the ovaries. It is planned to test this during the height of the breeding season, so as to discover whether this is a normal occurrence or whether the "shed" eggs are more likely to be "over-ripe" than the others, late in the season.

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[1] Loeb, Jacques, " Die chemische Entwicklungserregung, etc.," Julius Springer, Berlin, 1909.

[2] McClendon, J. F., " On Artificial Parthenogenesis of the Sea-urchin Egg," *Science*, 1909, Vol. 30, pp. 454–455.

[3] Glaser, O. C., " On Inducing Development in the Sea-urchin (*Arbacia punctulata*), Together with Considerations on the Initiatory Effect of Fertilization," *Science*, 1913, Vol. 38, pp. 446–450.

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BIOLOGICAL BULLETIN

SELECTION FOR HIGHER AND LOWER FACET NUMBERS IN THE BAR-EYED RACE OF DROSOPHILA AND THE APPEARANCE OF REVERSE MUTATIONS.[1]

H. G. MAY.

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The Experiments of Zeleny and Mattoon.

In 1915 Charles Zeleny and E. W. Mattoon published their results obtained by selecting for higher and lower facet numbers in the bar-eyed race of *Drosophila*. Three lines were selected for higher numbers and three lines for lower numbers. Individual pairs were mated and the selection was carried on for three generations in each direction.

They counted the facets in 250 flies at the beginning of the experiment and found the average number to be 98.04 for males and 65.06 for females. Counts made on 250 more flies from the stock at the end of the experiment gave the same results. From the ratio between numbers from males and females they computed a factor for converting the female number to the male number and published all numbers on the male standard. Without exception each generation produced successively higher numbers in the upward selected lines and successively lower numbers in the downward selected lines, and in each case the three lines were very close together. The average facet number was raised from 98.0 to 139.5 and lowered from 98.0 to 83.7. The maximum was raised from 182 to 213 and the minimum was lowered from 45 to 36. This increase in the range was not due to a larger number of counts, as 500 flies were counted from the stock and only 450 from the selected lines.

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[1] Contribution from the Zoölogical Laboratory of the University of Illinois, No. 97.

The Experiments of MacDowell.

In the same year E. Carlton MacDowell published the results of selections for higher bristle numbers in a race of *Drosophila* with extra bristles on the thorax. For six generations the number of extra bristles increased, but failed to rise any higher in forty additional generations. The response to selection was not so definite as in the experiments of Zeleny and Mattoon. The variation in the averages from different lines was very large. While the average of a large number of lines increased with each generation, that of any particular line often decreased for a generation. This wide range of fluctuations in the averages from different lines was partly explained by the existence of a correlation between the number of extra bristles and the size of the fly.

There is, however, another possible cause that was apparently overlooked. MacDowell selected for extra bristles in two arbitrarily limited rows on the dorsal side of the thorax, but states that even when no extra bristles were present in those rows "these flies frequently showed extra bristles on other parts of the thorax." The factors for extra bristles evidently controlled not only the number of extra bristles in those rows, but also the number of extra bristles on other parts of the thorax and possibly over the entire body. Selection, then, was made for only a small part of a variable character. A high number of bristles in the area under observation would in general indicate a high number of bristles on other parts of the body, but might actually be accompanied by a low number of extras elsewhere. Low offspring from high parents could be accounted for in that way. The efficiency of the selection would also decrease as the part of the character under observation decreased in comparison with the part not under observation.

The Present Experiments.

Since the experiments of Zeleny and Mattoon gave such clear results, but were interrupted before they led to a final conclusion, it seemed desirable to repeat them on a larger scale and continue them for a greater number of generations to determine if a pure line could be established and to study the changes in the existing factor or factor complex.

Material and Methods.

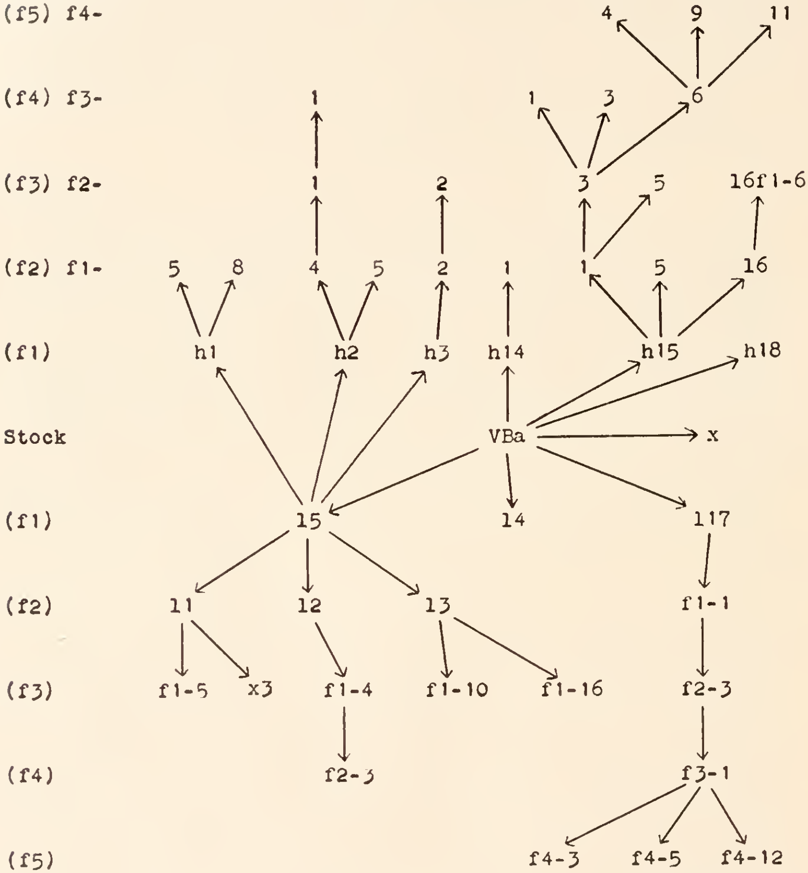
In order to have a check on any possible contamination a stock was selected which had a second recessive character, the vestigial wing, in addition to the bar eye. This stock also had the advantage of having on the average a lower facet number than the long-winged stock. But sterility in the race and the indefinite character of the results made it necessary, after a few generations, to return to the long-winged stock. In both cases the eye color was that of the normal wild fly.

The vestigial-winged, bar-eyed stock was designated as VBa; the long-winged, bar-eyed stock as Ba. The downward and upward selected lines in VBa were distinguished by l and h, in Ba by d and u respectively. Individual lines were distinguished by numbers. Any given mating received the number of the generation to which it belonged and a serial number corresponding to the number of matings made from that line in the given generation. In that way a number like Bau4f2–5, although cumbersome, gives nearly the whole pedigree of the mating involved. Since the lines are now clearly distinguished without the stock designations, Ba and VBa, those designations will frequently be omitted in this paper.

The material for the present set of experiments was obtained from Professor Charles Zeleny on whose advice the work was undertaken. To him the author is also indebted for a keen interest in the work and for many helpful suggestions.

All stocks were kept in large bottles while eight dram vials were used for individual pairs. The vials were plugged with cotton and in case of the long-winged race contained pieces of filter paper to prevent the flies from adhering to the food.

Throughout these experiments bananas were used as food. Only specimens with perfect skin were selected. The pulp was never allowed to come in contact with the outside of the skin


(f5) f4-
(f4) f3-
(f3) f2-
(f2) f1-
(f1)
Stock
(f1)
(f2)
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(f4)
(f5)
4
9
11
1
3
6
1
2
3
5
16f1-6
5
8
4
5
2
1
1
5
16
h1
h2
h3
h14
h15
h18
VBa
x
15
14
117
11
12
13
f1-1
f1-5
x3
f1-4
f1-10
f1-16
f2-3
f2-3
f3-1
f4-3
f4-5
f4-12

FIG. 1. Relation of selected lines and stock in VBa. In general the downward selected lines are below and the upward selected lines above.

and was heated to 70° C. and allowed to cool in a closed vessel before being placed into the food jar. Food was allowed to undergo alcoholic fermentation for about three days before being used. At first the author had considerable difficulty in keeping food, as acetic acid fermentation soon set in, the food became hard, and larvæ did not seem to thrive well in it. This defect, however, was remedied after one or two generations had been reared from the vestigeal-winged stock and long before the experiments on the long-winged stock were undertaken.

In some preliminary work the number of facets was estimated in the eyes of the parents selected and the actual counts were made at the time the parents were killed. This method was employed by Zeleny and Mattoon. The author, however, found that his estimates were not close enough and that in some cases parents were lost because they died and were destroyed by the larvæ before their death was discovered. The facets of dead flies also disintegrate when they remain in contact with food for a short time. For those reasons all selections during the experiments were made from actual counts.

During all of the work on the vestigial-winged flies and during part of the work on the long-winged flies, selections were made at noon and night after all the flies had been removed in the morning. In that way the specimens were never more than six hours old at the time of selection. But observations during that time showed that males seldom or never mated before they were twenty-four hours old, and for that reason later selections were made every twelve hours. All desirable specimens were saved and when no mates were present they were kept in vials with food until matings could be made.

All flies were etherized and the facets were counted while they were quiescent. The larvæ were never subjected to ether, as the flies to be examined were first transferred to empty vials. Selected flies recovered from the effect of the ether in fifteen minutes to half an hour. The other flies were preserved in 85 per cent alcohol.

For counting facets the flies were placed in a little pit on a paraffin block and illuminated by means of a 25-watt tungsten lamp. This gave sufficient light but only a moderate amount of heat. A Leitz microscope with a number four ocular and a number three objective and the tube drawn out to its full length was found to be most convenient. This gave a relatively high magnification with a suitable depth and size of field.

Errors in counting can not be avoided; it is merely a question of reducing them to a minimum. In eyes with 100 facets or less the error is certainly less than one per cent., but it increases rapidly as the facet number increases. Recounts made on eyes with as many as 300 facets showed an error of possibly two or three per cent. Of course it takes two or three weeks of practice to reach that point of efficiency.

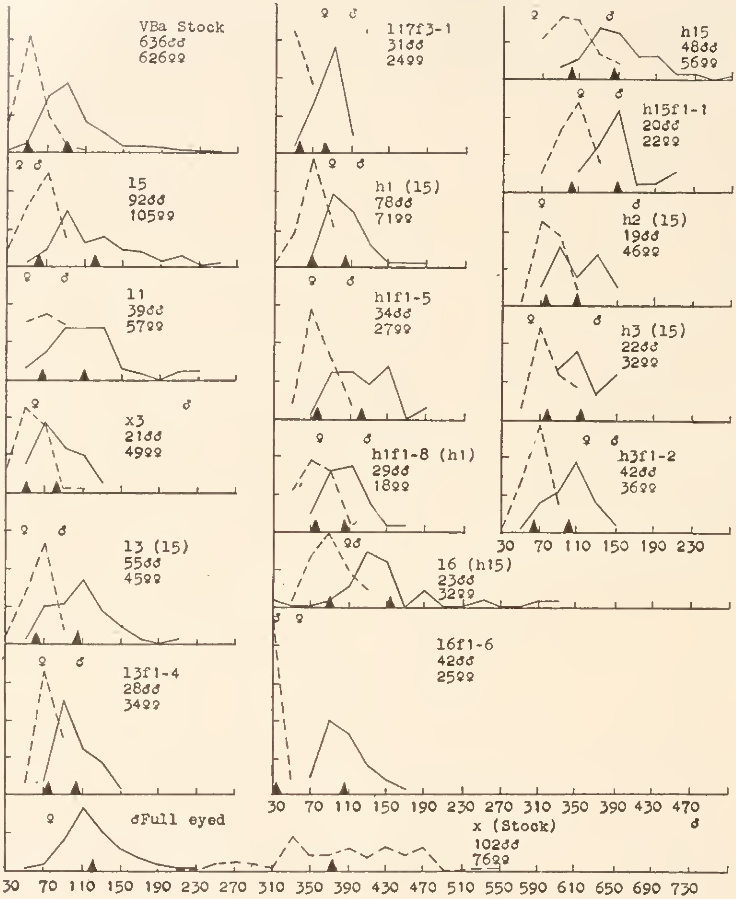

FIG. 2. Curves showing the effect of selection in individual lines in VBa. All curves are plotted on the scale of fifty. The males are represented by continuous lines and the females by interrupted lines. The parents are indicated at the top and the averages by the black pyramids below. The values plotted are for flies between 20 and 39, 40 and 59, 60 and 79, etc. The number of the mating is given above, followed by the source in brackets unless the parents come from the mating represented by the curve just above. In case of difficulty in determining the relationship of the lines consult Fig. 1.

Errors are due chiefly to the following causes: (1) The arrangement of the facets may be irregular. Ordinarily the facets are arranged in rows and one can easily count two rows at a time even if the eye is large. But in the bar eye the dorsal part usually contains a large number of facets without any indication of rows. This area increases very rapidly with the size of the eye. In heterozygous females the facets are all arranged in beautiful rows, and one is thus able to distinguish readily between the eye of a heterozygous female and a bar-eyed male even if both eyes contain the same number of facets. Bar-eyed females as a rule contain more regular facets than the males and the distinction between low heterozygous females and high bar-eyed females is more difficult. (2) There may be small facets either among the others or at the margins. These facets are most abundant in irregular eyes and may be one of the causes of irregularity. All grades are found from mere prominences that can only be seen with the most advantageous light to normal-sized facets. As a rule, however, there are few if any doubtful facets. (3) There may be colorless facets at the margins. Normally the colored area of the eye extends somewhat beyond the facet area, but in some rare cases a few facets may extend beyond it and these may be overlooked. (4) When the eye is so large that the fly must be turned to count all of the facets some of the facets in the middle may be recounted or omitted. All heterozygous females and some high grade males had to be turned. The error in this case also is not very great, as the bar eye as well as the heterozygous eye is almost divided in the middle. (5) Errors may also arise through a lack of concentration or through the inability to make the eye retain its position. These are purely personal elements.

No mechanical device was used for marking off the counted area from the uncounted area on the eye of the fly. The author relied entirely on the ability of his eye to follow the rows or, in the absence of rows, to mark off certain areas and hold them until the eye was counted. A cross-hair in the ocular was tried, but was found to be unreliable. It could be used if the eye of the fly presented a flat field, but with the rounded contour of the eye the hair keeps on traveling over facets as one focuses up and down and comes to rest on the same facets only in the same focal plane. But it is impossible to count all facets without large changes in the focus. In shifting the fly after a given area has been counted it is evident that the same shift makes the hair pass over more facets on a slanting area than on a horizontal one, just as surveyors will pass over more surface in surveying up or down a hill than on the level. But on the rounded eye the hair usually passes over both a horizontal and a slanting area at the same time. For that reason one can not possibly shift so that the hair crosses the same number of facets at every point on the eye. A cross-ruled ocular has little or no advantage over a cross-hair, and a camera lucida is scarcely worthy of consideration as it is difficult enough to keep it at the proper place even on a perfectly flat field.

The largest error, by far, is due to the fact that only the right eye was counted. Zeleny and Mattoon reported that they found the averages of a large number of counts to be the same for both eyes. The author obtained the same results. But that does not mean that both eyes in any given fly have the same number. Normally the variation is not more than about 1 per cent from the mean, but the author has found it as high as 5 per cent, and in one case 10 per cent. One abnormal male was obtained with 25 facets in the right eye and 146 in the left. It is obvious that the error is greatest in the parents and the extremes of the offspring as we are dealing in those cases with individual flies. In parents the left eye was usually examined to see that it was not abnormal, and actual counts were made in a large number of cases. The abnormal male was mated to see if the unequal condition would be inherited, but it died within twenty-four hours without giving any offspring.

Selections in the Vestigial-Winged Stock.

When the bottle that was to give rise to the VBa stock was received, flies were transferred to two fresh stock bottles and the offspring from these parents were used for making the original selections. From October 31 to November 5 some preliminary counts were made on flies that hatched in the original bottle. Sixty-nine males gave a mean facet number of 115 and seventy females gave a mean of 63.

All of the offspring from the parents placed into the new stock bottles were counted, including the ones that were used to continue the stock, and selections were made during the entire period. From the preliminary counts it was concluded that flies above a certain limit could be selected as high and those below another limit as low; but when the selections were begun it was found that no flies appeared that approached the lower limit set for high selections and many flies were below the limit set for low selections. As a result a new standard had to be established. Later the stock became more variable and some higher flies appeared together with some very low ones; indeed the lowest female obtained during these experiments was taken from one of these bottles. In all 370 males gave an average of 96 facets and 364 females gave an average of 54 facets. More counts on the stock were made March 17 to April 2, and this time 197 males gave an average of 82 facets and 192 females, an average of 44. This variability of the stock was contrary to the conditions found by Zeleny and Mattoon and seriously interfered with the success of the present experiments. It was necessary to pay as much attention to the nature and possible cause of the variation as to the effect of selection.

The greatest difficulty encountered in the VBa selections, however, was sterility and low production in the single lines. Of 42 matings made from the stock only 6 were fertile and of these only 2 gave a sufficient number of offspring for selection. On the whole about 80 per cent of the matings were sterile, and in many cases even lot matings failed to produce any offspring. By the end of the fifth generation all lines had died out except l17 and h15, and these were saved only through lots. For that reason the experiment was discontinued at that point.

No special experiments were made to determine the cause of sterility. Obviously it was not due to inbreeding, for it was as great in the first generation as in succeeding ones. In the majority of cases it was also not due to the inability of the females to deposit eggs as was the case in the experiments of Hyde. A few of such abnormal females were observed, but in most cases eggs appeared in the food and failed to hatch. The

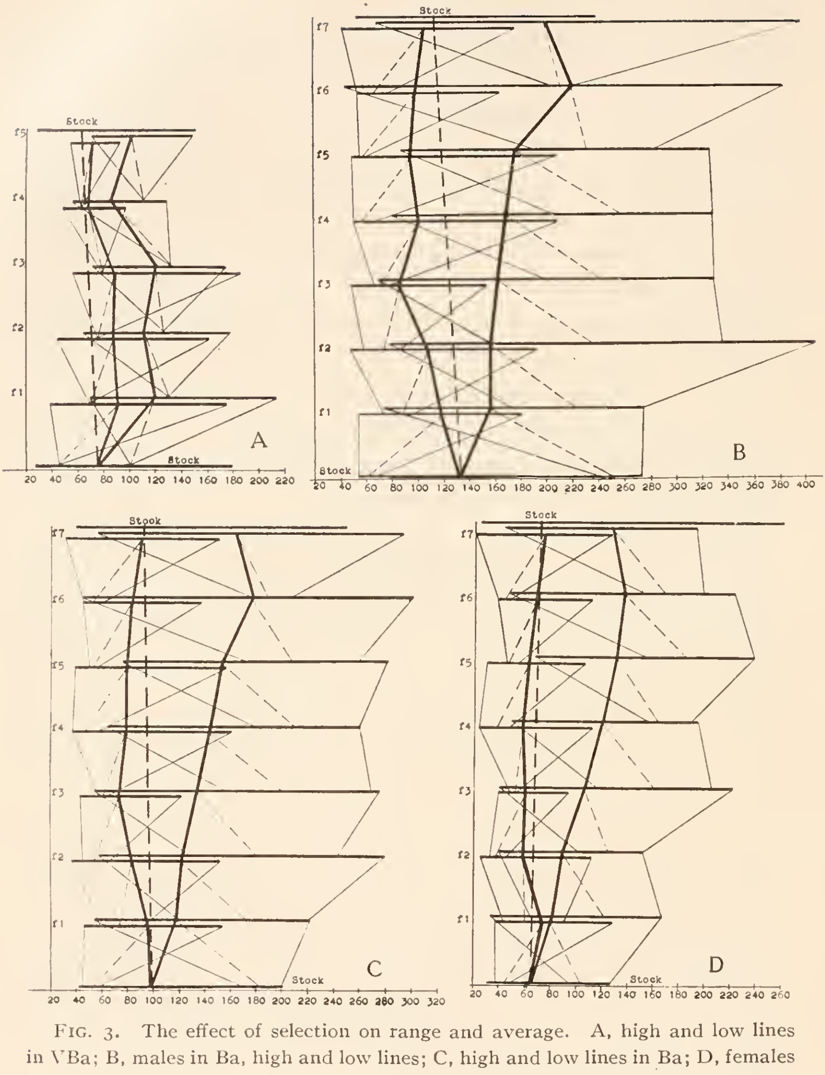

FIG. 3. The effect of selection on range and average. A, high and low lines in VBa; B, males in Ba, high and low lines; C, high and low lines in Ba; D, females in Ba, high and low lines. A and C are plotted for the means between male and female averages. These figures do not represent individual lines, but include all lines except reverse selections for the generations indicated. The heavy horizontal lines indicate the range, the heavy vertical lines, the averages. The heavy interrupted vertical lines merely connect the initial and final stock averages and do not indicate the averages of the stock at intermediate points. The fine slanting lines connect the extremes of the parents with those of the offspring while the fine interrupted lines connect the averages of the parents with those of the offspring. The facet numbers are indicated at the bottom, the generations at the left hand side.

results of Castle correspond more nearly to the facts observed.

The results obtained from these selections can best be made out by consulting Table I. and Figs. 1, 2 and 3.

The attempt to select downward was entirely unsuccessful. The selection was never very rigid, but the average of the offspring persistently remained above that of the stock.

The upward selections were more successful. In h15 the males rose from the stock average of 96 to 147 and the females from 54 to 101. In the other two lines where smaller numbers of offspring were obtained the rise was not quite so large. The second and third generations, however, failed to give any further increase in the facet number. Upward selections taken from line l5 after the first generation failed to give any rise in the facet number. The line x3 was taken from l1 after two generations of downward selection and gave a decrease in the facet number in spite of the fact that the male had 222 facets and was mated to average females.

The line l6 was intended to be a downward selection from h15, but the parents proved to be very near the mean and all matings from the first generation were sterile except f1–6. This particular mating involved a male with 34 facets, the lowest number obtained in these experiments. Fortunately a fair number of offspring was obtained. The males average much lower than those of the previous generation and about the same as those of the low lines hatching at the same time, but the females average much lower than anything obtained elsewhere. It is likely that the male with 34 facets was a mutant and that the mutation chiefly concerned the sex chromosome. The complete sterility of this race prevented any further investigation.

The results here obtained are very different from those reported by Zeleny and Mattoon. In their selections all three generations went gradually upward and gradually downward; in these experiments there was a sudden rise in the upward selected lines in the first generation and no further effect, and no response at all in the downward selected lines. For that reason and also on account of the high degree of sterility it seemed best to make some selections in the long-winged stock used by Zeleny and Mattoon.

TABLE I.

RELATION OF PARENTS AND OFFSPRING. VESTIGIAL-WINGED, BAR-EYED STOCK (VBa).

Parents.				Offspring.							
		Facets.		♂				♀			
Bottle Number.	Source.	♂	♀	No. Flies.	Facets. Ave.	Max.	Min.	No. Flies.	Facets. Ave.	Max.	Min.
Stock	Stock			636	93.8	253	36	626	51.9	108	19
l4	Stock	63	46	3	101.7	134	85	12	71.9	93	38
l5	Stock	60	45	92	120.2	254	56	105	63.2	98	23
l1	l5	86	48	39	110.3	222	57	46	67.4	95	41
l1f2	l1	64	46	10	116.5	269	77	12	73.8	104	56
l2	l5	56	37	13	107.3	142	70	14	69.2	88	52
l3	l5	98	53	55	106.3	208	56	45	63.6	88	32
l3f1–4	l3	108	71	28	102.9	149	76	34	74.7	97	51
l3f1–10	l3	96	53	9	116.1	142	82	10	77.6	96	56
l3f1–16	l3	75	56	1	121.0			1	86.0		
l3f2–3	l3f1–4	78	51	2	57.0	58	56	5	50.2	54	47
l17	Stock	46	19	2	98.0	108	88	2	85.5	90	81
l17f2	l17	108	90	4	118.8	122	116	8	92.4	102	83
l17f2–3	l17f2	116	83	12	100.9	165	70	17	60.1	89	42
l17f3–1	l17f2–3	113	81	31	85.0	117	57	24	56.3	76	41
l17f4–3	l17f3–1	77	54	9	84.2	96	70	3	64.0	74	51
l17f4–5	l17f3–1	lot	lot	16	82.4	108	60	11	57.2	77	48
l17f4–12	l17f3–1	lot	lot	2	90.0	100	80	6	59.8	65	53
h1	l5	120	86	78	106.2	182	71	92	70.1	99	29
h1f1–5	h1	108	74	34	122.0	192	78	27	77.7	101	58
h1f1–8	h1	128	75	29	107.3	160	75	18	73.6	103	52
h2	l5	170	73	19	110.7	163	68	46	79.4	105	53
h2f2	h2	156	90	5	146.2	175	125	6	89.3	113	79
h2f1–5	h2	163	100	4	88.0	112	72	6	58.7	69	48
h2f2–1	h2f2	150	113	2	103.5	115	92	6	59.7	70	50
h2f3–1	h2f2–1	115	70	14	109.6	143	88	13	67.4	89	55
h3	l5	126	64	22	114.8	154	82	32	80.0	116	57
h3f2	h3	145	116	42	102.7	150	52	36	67.4	95	29
h3f2–2	h3f2	122	90	3	80.3	85	72	1	65.0		
h14	Stock	155	64	9	128.3	190	95	3	89.0	108	77
h14f2	h14	161	108	4	94.0	113	74	9	69.0	82	63
h15	Stock	137	60	48	147.1	271	81	56	101.2	158	70
h15f1–5	h15	123	96	17	121.7	154	84	14	83.6	113	56
h15f2	h15	148	112	20	149.7	219	105	22	102.5	137	64
h15f2–3	h15f2	lot	lot	16	147.2	199	92	19	91.4	125	51
h15f2–5	h15f2	205	111	16	151.6	217	118	8	94.0	129	57
h15f3–1	h15f2–3	156	109	10	96.7	126	76	8	73.5	123	59

Parents.				Offspring.							
				♂				♀			
Bottle Number.	Source.	Facets.		No. Flies.	Facets.			No. Flies.	Facets.		
		♂	♀		Ave.	Max.	Min.		Ave.	Max.	Min.
h15f3-3	h15f2-3	150	107	3	84.0	97	62	3	76.7	85	61
h15f3-6	h15f2-3	158	102	9	97.0	134	63	19	73.8	103	50
h15f4-4	h15f3-6	131	103	7	120.6	196	79	4	89.3	100	65
h15f4-9	h15f3-6	lot	lot	3	117.3	144	88	3	78.7	87	63
h15f4-11	h15f3-6	134	93	2	124.0	131	117	3	74.3	86	63
h18	Stock	163	62	12	123.8	241	90	11	94.8	129	59
l6	h15	122	108	23	151.3	338	34	32	89.6	127	56
l6f1-6	l6	34	64	42	105.1	170	70	25	33.5	56	25
x	Stock	full	43	102	122.3	228	56	76	377.9	543	218
x3	l1	222	lot	21	82.0	127	56	49	54.9	104	31
x4	l6	328	lot	12	142.0	243	70	16	77.4	116	53
x4f1-1	x4	140	116	5	146.0	181	130	10	111.5	135	83
x4f2-5	x4f1-1	132	103	6	169.5	196	143	1	119.0		

SELECTIONS IN THE LONG-WINGED STOCK.

These experiments were begun on January 15 and continued until the middle of June. In this case the number of flies counted from the stock during selection was 91 males with an average facet number of 132 and 81 females with an average of 66. The first selection made was very near the mean and was designated as Ba1 and used as part of the stock. It yielded 44 males and 37 females with mean facet numbers of 125 and 68 respectively. In June the eyes of 73 additional males and 97 additional females from the stock were counted, yielding mean facet numbers of 114 and 72 respectively. Here again, as in the vestigial-winged stock, there is a change in the mean facet number, and in this case it also involves a decided change in the ratio between male and female facet numbers.

Little difficulty was encountered on account of sterility in this stock. About 75 per cent of the matings were fertile and nearly 75 per cent of the fertile matings produced 50 offspring or more. In each case all of the offspring were counted. To avoid the dying out of lines a number of branches of each line was run, only brothers and sisters were mated, but no attention was paid to the dying out of any given branch when it did not come up to the standard in facet number or production. In that way the most suitable flies from the most suitable branches were selected, bringing the selections to some extent on the behavior basis. By that method parents whose somatic constitution varies from their germinal constitution are to some extent eliminated.

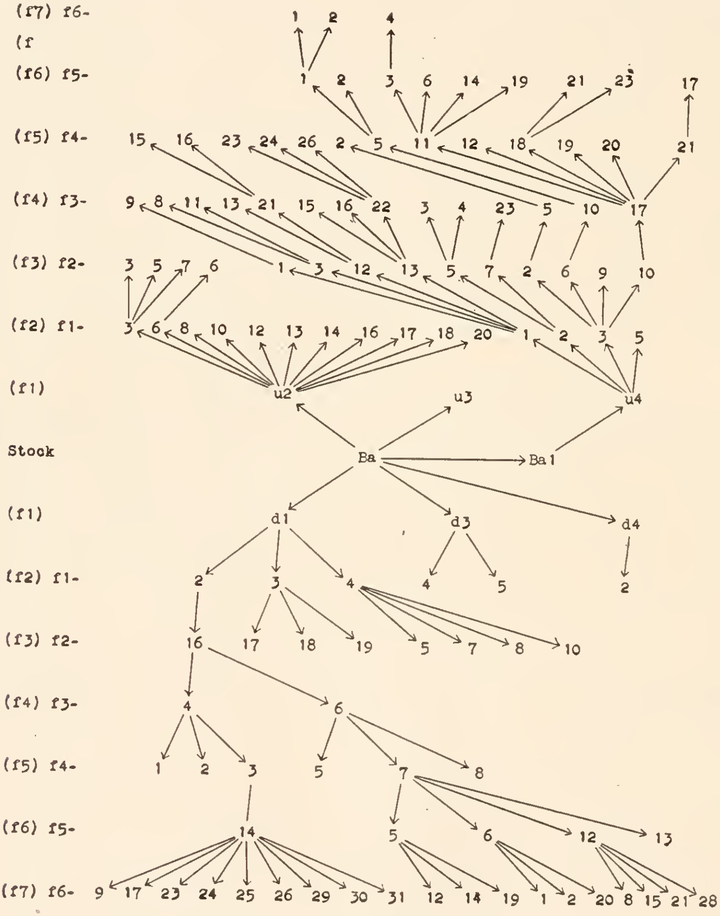
(f7) f6-
1 2 4
(f
(f6) f5-
1 2 3 6 14 19 21 23 17
(f5) f4-
15 16 23 24 26 2 5 11 12 18 19 20 21
(f4) f3-
9 8 11 13 21 15 16 22 3 4 23 5 10 17
(f3) f2-
3 5 7 6 1 3 12 13 5 7 2 6 9 10
(f2) f1-
3 6 8 10 12 13 14 16 17 18 20 1 2 3 5
(f1)
u2 u3 u4
Stock
Ba Ba 1
(f1)
d1 d3 d4
(f2) f1-
2 3 4 4 5 2
(f3) f2-
16 17 18 19 5 7 8 10
(f4) f3-
4 6
(f5) f4-
1 2 3 5 7 8
(f6) f5-
14 5 6 12 13
(f7) f6-
9 17 23 24 25 26 29 30 31 12 14 19 1 2 20 8 15 21 28

FIG. 4. Relation of selected lines and stock in Ba.

Selections in this stock were carried on for seven generations in each direction, several return selections were made, and crosses in both directions were finally made from the selected lines. The results are shown in Table II. and in Figs. 3, 4, 5, 6 and 7.

The downward selections were not very effective. In the first generation the average facet number in the females actually rose above that of the stock, but the average of the males dropped considerably below. In the next generation the females dropped decidedly and the males dropped only slightly. The third generation produced another slight lowering of the means, and after that there was a gradual return toward the mean of the stock.

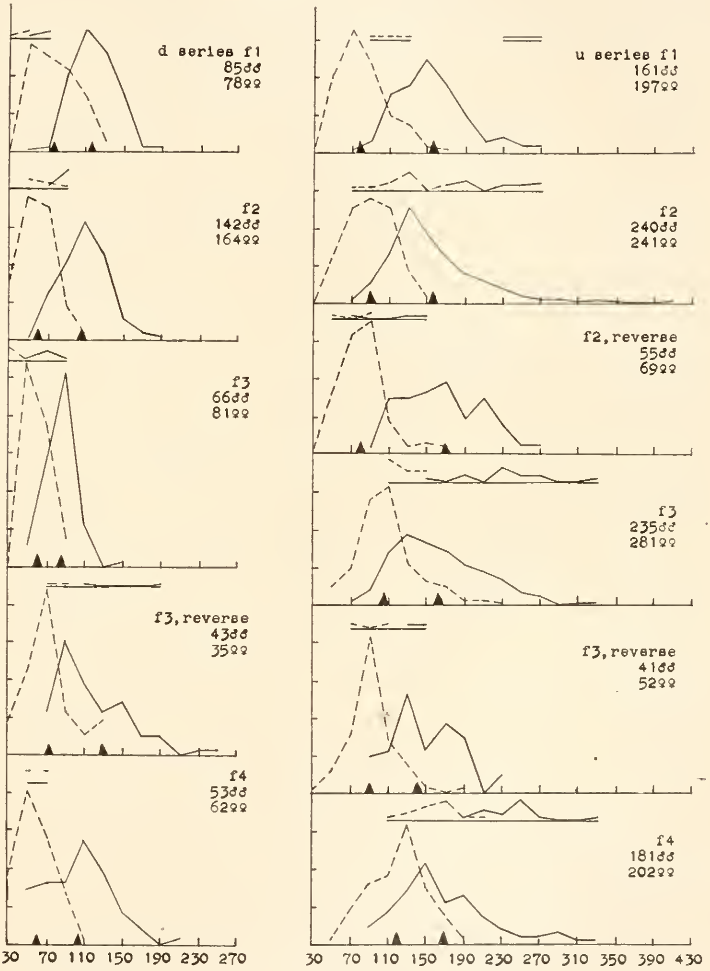

FIG. 5*A*.

In the seventh generation the mean of the stock had been reached by the females, but the males were still slightly lower. Taking the mean of the male and female averages more uniform results appear to have been obtained. In that case the first generation shows no change, the second and third generations show a gradual

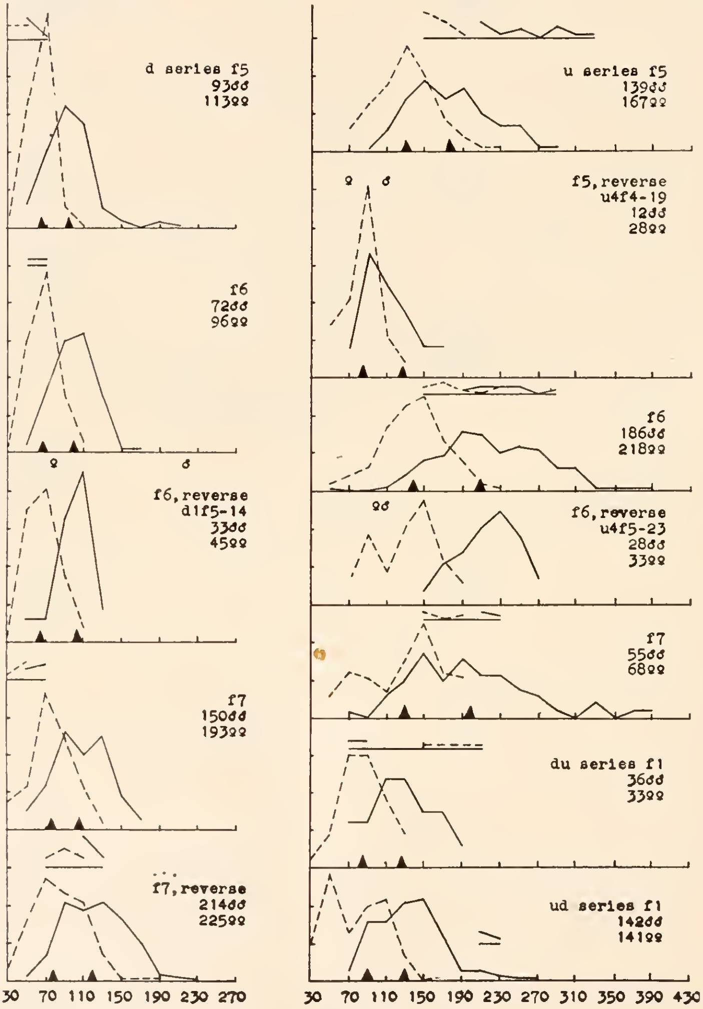

FIG. 5*B*.

FIG. 5. Curves showing the effect of selection for generations in Ba. These curves are plotted on a scale of one hundred. Parents are indicated by smaller curves at the top. In other respects the curves are like those of Fig. 2.

lowering, but the next four generations show a complete return to the mean of the stock.

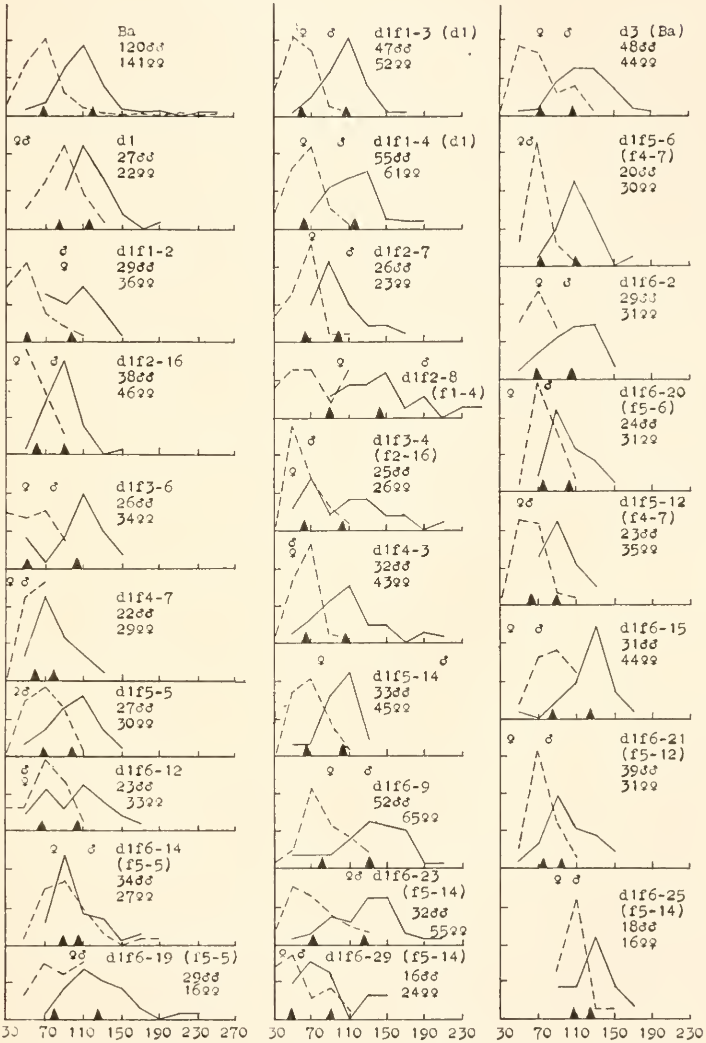

FIG. 6. Curves showing the effect of selection in individual lines in Ba, downward selections. For other information consult Fig. 2.

The upward selections were more successful. With the exception of the last generation there is in every case a slight rise in both sexes. The results in the last generation can be explained by the fact that the selections were below the average

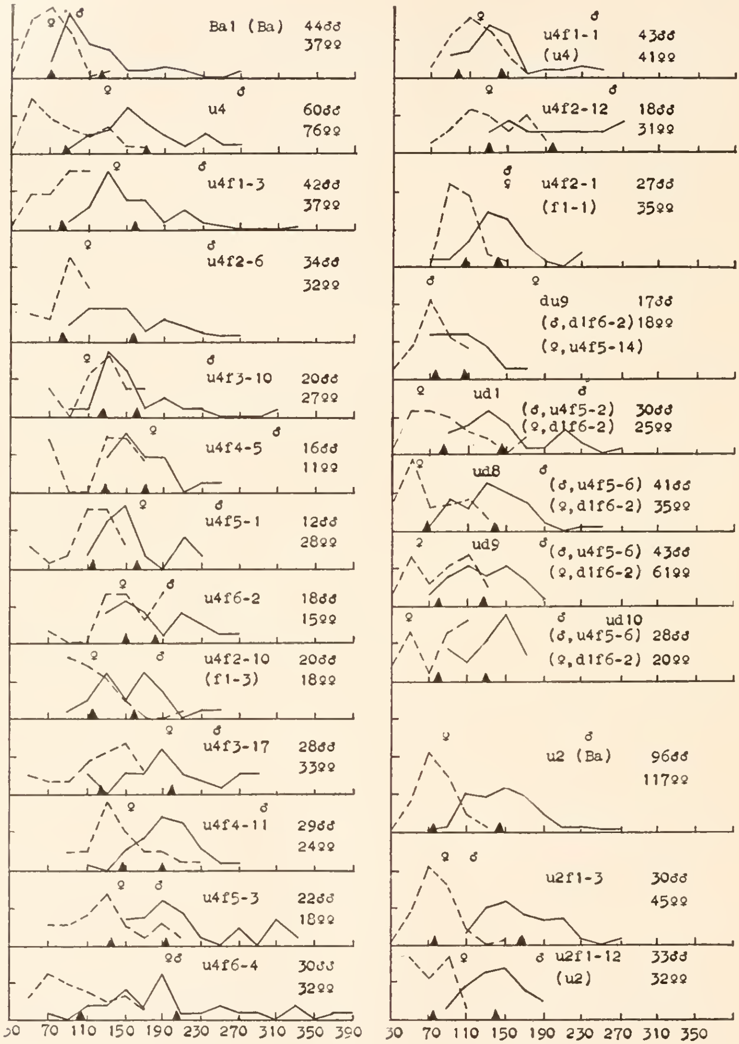

FIG. 7. Curves showing the effect of selection in individual lines in Ba, upward selections and crosses. For other information consult the description of Fig. 2.

in the males and not far above in the females, and the fact that the offspring hatched at a time when all counts were low. This will be explained later.

Table II.

Relation of Parents and Offspring. Long-Winged, Bar-Eyed Stock (Ba).

Parents.				Offspring.							
				♂				♀			
Bottle Number.	Source.	Facets.		No. Flies.	Facets.			No. Flies.	Facets.		
		♂	♀		Ave.	Max.	Min.		Ave.	Max.	Min.
Ba	Stock			120	122.7	240	53	141	69.1	259	24
Ba1	Stock	104	74	44	125.0	273	69	37	68.3	128	34
d1	Stock	53	38	27	117.4	180	91	22	84.0	122	56
d1f1–2	d1	92	91	29	99.5	141	64	36	52.5	102	25
3	d1	92	56	47	106.0	164	58	52	58.4	108	28
4	d1	98	65	55	114.7	192	62	61	59.4	111	32
d1f2–5	d1f1–4	84	62	0				1	65.0		
7	4	106	73	26	97.9	162	62	23	62.6	117	37
8	4	190	98	17	148.6	242	85	12	90.0	131	47
10	4	87	47	11	87.1	104	67	11	63.4	87	48
16	2	76	36	38	87.6	153	58	46	61.6	92	40
17	3	75	36	12	72.3	95	49	17	54.5	71	38
18	3	68	35	4	80.0	87	73	6	63.7	73	58
19	3	58	33	1	109.0			1	64.0		
d1f3–1	d1f2–10	76	48	2	88.5	90	87	2	50.0	53	47
4	16	68	54	25	103.2	209	51	26	60.6	111	26
6	16	79	53	26	101.8	154	57	34	55.1	98	23
d1f4–1	d1f3–4	57	48	11	98.9	122	74	7	64.6	68	59
2	4	51	44	11	90.2	107	79	13	70.2	88	49
3	4	55	47	32	109.4	208	55	43	62.2	104	37
5	6	59	32	4	87.0	96	75	6	66.8	82	37
7	6	57	23	22	80.3	133	56	29	59.1	77	29
8	6	69	35	13	91.2	115	50	15	63.5	91	49
d1f5–5	d1f4–7	56	45	27	98.9	155	54	30	70.8	100	37
6	7	61	46	20	111.5	165	78	30	72.3	105	45
12	7	56	55	23	90.2	127	65	35	61.3	110	39
13	7	62	51	2	78.5	83	74	1	71.0		
14	3	208	77	33	101.1	132	56	45	66.6	116	30
d1f6–1	d1f5–6	165	105					1	67.0		
2	6	102	70	29	108.2	155	59	31	69.7	99	42
8	12	126	110	2	102.5	103	102	3	85.0	90	81
9	14	125	93	52	131.5	215	50	65	82.0	139	27
12	5	54	49	23	103.7	166	42	33	69.3	104	22
14	5	122	83	34	103.3	164	69	27	93.8	187	52
15	12	68	45	31	123.4	167	51	44	86.1	114	44
17	14	119	92	1	121.0						
19	5	111	100	29	127.3	237	71	16	84.6	117	52
20	6	78	45	24	101.9	144	72	31	78.9	112	49
21	12	78	39	39	97.4	153	51	31	72.7	104	40
23	14	116	110	32	125.3	206	51	55	70.9	135	23

TABLE II.—*Continued.*

Parents.				Offspring.							
		Facets.		♂				♀			
Bottle Number.	Source.	♂	♀	No. Flies.	Facets. Ave.	Max.	Min.	No. Flies.	Facets. Ave.	Max.	Min.
d1f6–24	14	114	94	13	135.5	185	81	16	104.2	127	73
25	14	113	90	18	125.1	163	89	16	107.5	145	86
26	14	108	66	20	105.0	147	51	7	87.3	121	49
28	12	77	48	14	127.1	176	72	24	88.0	125	30
29	14	57	43	16	90.8	158	56	24	53.9	109	20
30	14	117	72	13	115.5	178	78	19	65.8	105	42
31	14	56	30	3	88.3	95	83	6	71.2	90	46
d3	Stock	65	51	48	118.2	181	54	44	72.8	128	37
d3f1–4	d3	74	43	1	92.0			7	72.0	92	54
5	d3	84	45	2	59.0	70	48	1	45.0		
d4	Ba1	69	48	10	116.3	145	81	12	66.9	86	50
d4f1–2	d4	90	61	8	128.6	157	108	5	64.2	78	54
u2	Stock	238	94	96	147.6	268	74	117	77.5	132	39
u2f1–3	u2	122	89	30	165.7	261	103	45	75.2	141	32
6	u2	144	94	15	187.7	249	107	14	90.9	162	52
8	u2	172	92	28	172.1	408	79	26	90.2	148	59
10	u2	187	132	22	191.8	330	125	14	104.1	144	69
12	u2	190	111	33	141.6	191	86	32	74.7	113	43
13	u2	192	109	16	144.6	203	90	18	94.4	135	49
14	u2	220	110	2	175.5	186	165	4	116.5	130	105
16	u2	246	123	8	126.3	153	106	10	95.1	111	75
17	u2	268	126	5	206.6	278	130	4	116.3	124	110
18	u2	172	63	22	141.7	249	78	38	81.0	138	49
20	u2	74	56	10	127.1	221	90	10	90.5	100	77
u2f2–3	u2f1–3	190	102	15	157.0	240	88	16	91.3	116	72
5	3	261	146	1	136.0			2	121.5	134	109
6	6	220	114	4	114.0	120	104	2	81.0	88	74
7	3	136	76	6	107.7	138	81	6	77.0	88	68
u3	Stock	240	109	5	124.8	138	98	4	101.8	120	93
u4	Ba1	273	128	60	170.9	275	99	76	85.3	167	34
u4f1–1	u4	241	121	43	146.3	255	83	41	94.5	151	49
2	u4	271	160	18	154.2	274	100	15	105.5	140	66
3	u4	227	136	42	158.2	336	96	37	83.1	116	39
5	u4	275	167	1	161.0			2	100.5	118	83
u4f2–1	u4f1–1	153	151	27	141.4	231	74	35	106.0	173	61
2	3	147	106	35	153.3	221	93	46	92.1	191	35
3	1	225	116	51	151.6	331	70	41	87.3	144	40
5	2	223	140	13	179.4	224	112	11	110.0	173	51

TABLE II.—*Continued.*

	Parents.			Offspring.							
Bottle Number.	Source.	Facets.		♂				♀			
				No. Flies.	Facets.			No. Flies.	Facets.		
		♂	♀		Ave.	Max.	Min.		Ave.	Max.	Min.
u4f2–6	3	237	111	34	156.9	263	83	32	86.4	113	40
7	2	274	129	31	166.1	238	107	54	109.9	217	45
9	3	336	100	8	179.1	229	150	11	108.3	169	85
10	3	187	118	20	156.8	244	99	18	114.8	205	81
12	1	255	127	18	199.1	266	126	31	131.0	190	66
13	1	252	122	13	203.4	267	119	28	126.3	221	69
u4f3–3	u4f2–5	215	173	3	135.7	165	99	6	147.0	193	84
4	5	213	147	7	160.4	182	125	14	124.9	166	61
5	2	197	121	30	173.1	330	92	23	112.1	154	75
8	3	331	173	7	142.9	198	108	2	151.5	166	137
9	1	231	143	3	148.0	190	117	6	124.7	158	113
10	6	241	112	20	159.2	308	99	27	126.5	176	64
11	3	242	136	8	156.5	207	129	7	115.3	127	96
13	12	260	190	12	194.7	291	140	14	126.2	150	89
15	13	245	172	4	149.5	185	113	14	114.6	148	83
16	13	221	172	9	139.3	167	112	6	102.0	136	70
17	10	244	205	28	201.0	298	108	33	122.2	168	48
21	12	255	179	18	175.5	294	132	16	122.0	167	65
22	13	255	145	15	178.9	244	99	25	115.6	190	67
23	7	216	145	17	127.1	281	82	9	94.3	149	75
u4f4–2	u4f3–5	330	154	22	159.3	260	88	20	99.7	141	67
5	10	308	176	16	169.9	240	129	11	125.6	173	69
11	17	290	157	29	191.6	269	101	24	145.3	236	81
12	17	298	158	5	144.4	168	120	4	129.5	141	125
15	21	203	156	2	165.5	167	164	7	114.0	137	77
16	21	220	165	4	155.0	187	138	4	138.5	158	129
18	17	284	156	15	190.6	284	106	27	134.2	237	77
19	17	109	67	12	128.9	192	77	28	84.1	127	52
20	17	250	155	3	153.7	196	109	6	120.2	141	86
21	17	209	152	11	186.0	256	132	8	141.1	195	84
23	22	214	163	13	173.7	257	125	17	123.2	160	78
24	22	244	190	17	186.8	327	130	37	136.1	182	85
26	22	209	161	2	120.0	121	119	2	131.5	135	128
u4f5–1	u4f4–5	238	173	12	158.3	222	111	28	113.3	153	46
2	5	240	167	14	182.2	235	118	10	135.3	162	76
3	11	189	153	22	195.6	302	123	18	133.9	209	76
6	11	213	151	42	225.2	384	45	37	127.6	177	65
14	11	249	182	43	247.0	367	143	50	137.7	198	72
17	21	205	162	31	242.2	354	151	28	148.5	201	58
19	11	231	236	14	215.7	303	140	40	151.0	221	92
21	18	284	237	8	192.5	210	170	7	151.3	185	103
23	18	106	98	28	217.2	278	151	33	130.4	186	64
u4f6–1	u4f5–1	222	153	7	213.3	260	116	21	152.2	191	81
2	1	201	149	18	184.1	262	128	15	149.3	191	76
4	3	209	196	30	204.4	398	69	32	99.1	174	43

TABLE II.—*Concluded.*

Parents.				Offspring.							
				♂				♀			
Bottle Number.	Source.	Facets.		No. Flies.	Facets.			No. Flies.	Facets.		
		♂	♀		Ave.	Max.	Min.		Ave.	Max.	Min.
du1	6–2 4–11	67	200	12	148.3	198	116	9	98.0	122	67
2	5–12 5–2	82	151	6	146.8	178	110	5	95.4	124	60
8	6–2 5–3	88	171	1	135.0			1	112.0		
9	6–2 5–14	74	182	17	104.6	160	66	18	73.5	113	39
ud1	5–2 6–2	235	59	30	149.1	264	81	25	83.8	166	32
8	5–6 6–2	205	56	41	138.2	245	61	35	69.1	123	31
9	5–6 6–2	205	56	43	127.1	187	72	61	81.3	129	30
10	5–6 6–2	211	54	28	132.0	169	85	20	80.1	113	28

TABLE III.

DAILY COUNTS, CROSSES BETWEEN LOW MALES AND HIGH FEMALES.
(Males from d1, Females from u4.)

Date.	Badu1.				Badu2.				Badu8.		Badu9.			
	♂		♀		♂		♀		♂	♀	♂		♀	
	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.			Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.
My 24					117		124 110							
25	198 133 147				149 110 166									
26	163		122		178					112				
27	157 186	134	121 99		161				135		136 113		100 84 89	
Je 6	131 117 158 139 116		76 67 114 97	89 97				60 97 86			148 134 110 160 99 111 102 95 123 93	67 82 68 71 66	39 113 86 69 60 92 63 54 61 52 69 103 70 70	49

Table IV.

Daily Counts, Crosses between High Males and Low Females.
(Males from u4, Females from d1.)

Date.	Baud1.				Baud8.				Baud9.				Baud10.	
	♂		♀		♂		♀		♂		♀		♂	♀
	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.	Bot. 1.	Bot. 2.		
My 24	159													
	148													
25	200		133		144		121		158		104			
	238		161		138		112		121		126			
	264						116				98			
	224										115			
											113			
26	164		166		163		119		149		116		152	93
	211		118		125		123		179		96		169	102
	203		123		133		104		187		120		168	96
					153				175		113		120	
					143				174		98		127	
					136				144		119		157	
					136				116		105		133	
											109			
											127			
27	133		106		177		89		172		111		161	93
	184		105		169				169		122		161	113
	136		71		155				183		98		144	106
	132		82		245				178		116		131	113
	205		80		138				153		112		146	112
	129				198				153		111		155	112
					142						113		149	113
					153						80		146	
											108			
											129			
											124			
Je 7, 8	104	147	84	58	166	92	49	34	102	97	66	43	94	28
	81	114	54	64	239	123	57	80	150	72	33	75	115	49
	126	133	83	99	158	107	57	76	140	116	54	85	140	88
	94	108	65	32	111	96	51	62	128	85	32	81	96	42
	154	141	72	40	151	170	77	45	91	135	53	51	139	83
	97	118	54	52	163	108	46	77	109	118	65	30	140	37
	136	101		56	96	98	47	54	72	76	41	47	128	70
		89		63	83	125	88	46	81	113	35	48	109	52
				73		114	87	52	127	121	60	81	89	44
						Full		41	92	103	66	81	104	56
						134		34	121	105	68	51	87	
						126		31	150	83	55	40	85	
						61		48		111	109	55	150	
						94		104		141	85	99		
						95		87		92	37	71		
						114		37		123	80	91		
						195		32			48	58		
								36			57	43		
								100						

Return selections from the low lines produced on the average slightly higher numbers than low selections in the same generations. Individual cases, however, do not seem to show any definite response. The few return selections made from the high lines were even less definite. Only one gave offspring that could be distinguished from those of the generation in which they hatched (u4f4–19).

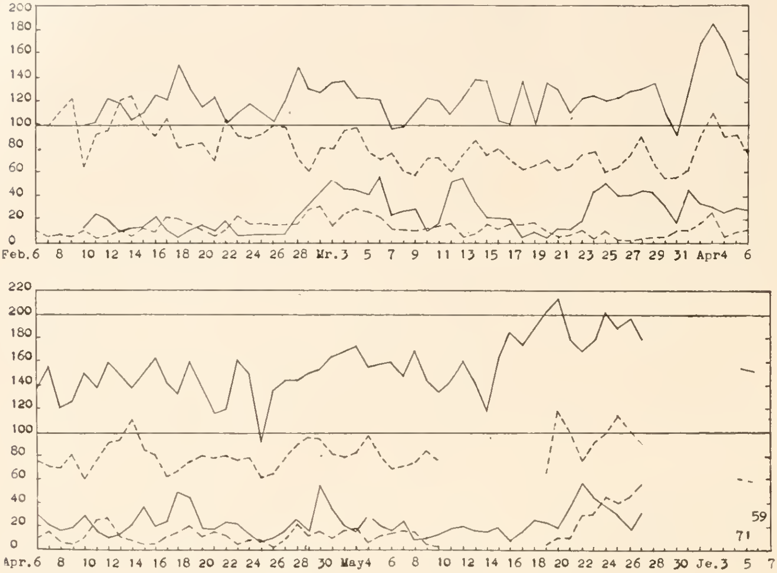

FIG. 8. Daily averages of high and low lines in Ba from February 6 to June 7, 1916. The high lines are represented by continuous lines and the low ones by broken lines. The two upper lines show the daily facet averages while the lower ones represent the actual numbers of flies from which those averages were obtained.

The offspring from the crosses between low and high lines are intermediate and show a very slightly wider range than the low lines, but not nearly so wide a range as the high lines. There is no significant difference between the offspring from high males and low females and those from low males and high females. The apparent difference in the extremes is accounted for by the fact that the standard of all the lines changed during the period of hatching. The results of these matings are given in actual daily counts in Tables III. and IV., and are also shown in Figs. 5 and 7. A comparison of Tables III. and IV. with Fig. 8 shows that the lower values in the later counts are entirely in agreement with the results obtained at this time in the high and low lines.

Such changes in facet numbers of all the lines had already been noticed in the vestigial-winged race and are clearly shown at several points in Fig. 8. The changes would appear more sudden and the agreement between the lines would be closer if it were not for the fact that the change appeared sooner in bottles that just began to hatch than in older bottles.

THE APPEARANCE OF HETEROZYGOUS FEMALES AND FULL-EYED MALES.

What developed to be the most puzzling phase of the entire set of experiments was the appearance of full-eyed males and heterozygous females in the stock bottles and in the selected lines during the period they were under observation. Eleven such individuals appeared at different times as shown in Table V.

TABLE V.

PEDIGREE OF FULL-EYED MALES AND HETEROZYGOUS FEMALES OBTAINED FROM BAR-EYED STOCK UNDER OBSERVATION.

Sex.	Date of Appearance.	Line.	Facets.	Fate.	Grade of Mates.	Offspring.				
						Bottle No.	♂		♀	
							Bar.	Full.	Bar.	Het.
♂	Nov. 15, 1915	VBa (Stock)	700?	Mated	43	VBax	102			76
♂	Nov. 29, 1915	VBa (Stock)	1200?	Died						
♂	Dec. 17, 1915	VBah15	1200?	Sterile						
♀	Jan. 16, 1916	VBah3f2	371	Mated	109 116	VBah3f2–6	4	4	1	4
♂	Feb. 12, 1916	Bau2	1200?	Mated	61 67	Bau2x	13			10
♀	Mar. 10, 1916	Bau2f2–3	601	Died						
♂	Mar. 28, 1916	Bau4f2–10	700?	Mated	149	Bau4f3x	27	17[1]	20[1]	18
♀	Apr. 2, 1916	Bau4f2–13	481	Mated	238 204	Bau4f2–13x	3	3	2	4
♀	Apr. 28, 1916	Bau4f4–16	621	Mated	187	Bau4f5x	11	13	7	11
♀	June 6, 1916	Bau4f6–4	452	Killed						
♂	June 7, 1916	Baud8	800?	Killed						

[1] The full-eyed males and bar-eyed females in Bau4f3x were undoubtedly due to the appearance of flies from the second generation before the counts were made. The parents were mated April 3 and by an oversight the offspring were permitted to remain in the bottle until April 27.

The VBa stock in March revealed no such specimens, but the Ba stock in June contained several of each. In that case they were probably due to the appearance of a heterozygous female in the previous generation. One of the stock bottles received from Professor Zeleny contained a mixture of bar-eyed, full-eyed and heterozygous individuals. These may have been due to the appearance of a full-eyed male or a heterozygous female at some previous time.

The eyes of the males could not be distinguished from those of normal wild flies. The facet numbers indicated in the table are mere estimates obtained by counting a row of facets to the middle of the eye and using the number obtained as a radius for computing the number on the entire area. The eyes of wild flies treated in the same way gave similar results. The eye of a wild male which could be counted without turning was found to contain 700 facets.

The eyes of the heterozygous females had the characteristic appearance of the eyes of such females obtained from crosses between wild and bar-eyed flies and contained the corresponding facet numbers. The ordinary bar eye has the shape of a crescent with a notch near the middle of the concave side almost separating the two ends of the crescent. One end is usually considerably larger than the other and frequently contains the irregular facets mentioned before. The eye of the heterozygous female has the shape of the bar eye, but is much larger and does not contain the irregular facets. As shown in the table, the heterozygous females from the long-winged race had considerably larger eyes than those from the vestigial-winged race. This is explained by the fact that the average facet number in the bar eye of the long-winged race is much larger than that of the vestigial-winged race.

Wherever possible the full-eyed males and heterozygous females that appeared were mated, but, with the exception of the first case, no attempt was made to get a large number of offspring or to count the facets in the bar-eyed and heterozygous offspring. Two of the individuals died soon after hatching and the last two could not be mated because the experiments had to be brought to a close. Of those mated one proved to be sterile and the other six produced offspring. In each case the specimens were mated with bar-eyed flies and with one exception the full-eyed males produced nothing but bar-eyed males and heterozygous females and the heterozygous females produced bar-eyed and full-eyed males and bar-eyed and heterozygous females. The one exception occurred in case of the male which appeared on March 28 and was mated to a bar-eyed female on April 3. The bottle was set aside and overlooked until April 27 when it was found to contain all four classes of flies as shown in the table. In that case the full-eyed males and bar-eyed females are undoubtedly due to the appearance of flies of the second generation before the count was made. Since the first generation consisted of bar-eyed males and heterozygous females the second generation would be expected to contain a mixture of all four classes.

The facets in the eyes of all the offspring obtained from the male appearing on November 15 were counted and the results are given under VBax in Table I. and Fig. 2. A large number of pairs from these offspring was mated, but all proved to be sterile. A mass mating made toward the end of the hatching period, however, produced 14 bar-eyed males with an average facet number of 114, 7 full-eyed males, 12 bar-eyed females with an average facet number of 91 and 5 heterozygous females. These were bred in a stock bottle for several generations to see if one or the other class would become dominant, but no obvious change in the ratios appeared.

Discussion and Conclusions.

The variability of the stock at the beginning of the selections made it appear probable that the facet number is affected by environmental conditions. For that reason the records during the experiments were kept more carefully than would otherwise have been the case. Counts were made and recorded separately at least every twelve hours. Each line or branch of a line was recorded separately, and in cases where parents were transferred and offspring obtained from both bottles a separate record was kept of each. Observations were also made on a possible correlation between body size and facet number.

It was impossible to find any noticeable effect of moisture, consistency, or age of food on the facet number. No significant difference was found in facet number between the first flies hatching from a bottle and the last ones except in cases where the food gradually dried up and the last flies were minute, *i. e.*, one half the size of normal flies or less. Such minute flies showed a tendency toward lower facet numbers. Accurate size measurements and facet counts on a large number of flies may possibly reveal a slight correlation between size of body and number of facets, but mere observation failed to detect it. It is also possible that a slight correlation exists but is concealed by other factors that have more influence.

No relation could be discovered between the age of the parent and the facet number of the offspring. In case of such a relation the latest offspring from two parents would be different from the first. No such difference could be detected.

Likewise it was impossible to find any definite correlation between the length of the developmental period of the larva and the facet number of the adult. No special experiments were undertaken with material in which the exact developmental period was ascertained, but, since in productive bottles the period of hatching is much longer than the period during which the eggs are laid, the first larvæ must as a rule have a shorter developmental period than the last, and any difference due to the length of the developmental period should become evident.

A glance at Fig. 8 shows that beyond the daily fluctuations due to the fact that the number of flies examined was too small to be representative, there are larger, parallel fluctuations of the two lines. Since the food during the Ba selections was fairly constant in character it can not be regarded as the cause of these fluctuations. The temperature of the room in which the flies were kept varied between rather wide limits. Unfortunately no thermograph was available for the room at that time. There is no correlation between the outside temperature and the facet number; but the variations in the room temperature were independent of those in the outside temperature. Since experiments to determine the effect of temperature on the facet number were undertaken by E. W. Seyster of this laboratory during the latter part of this work and on account of the lack of time no special effort was made to determine the exact relation between temperature and facet number. The fact that the change usually occurred one to three days sooner in bottles that just began to hatch than in old ones can be explained by the assumption that the effect was produced at an early stage in the developmental period. Since the first flies hatching from a bottle must have a shorter developmental period than the later ones they would be the first to show the effect.

It does not seem possible, however, that temperature is the sole cause of somatic variations. In the VBa selections it was observed that the two lines throughout averaged higher than the stock. In spite of that fact an examination of the extremes shows that most of the high flies were eliminated from the low lines. The rank of the average flies must, then, have been raised. The same can be said of the low lines in Ba. Here the elimination of high flies is much more pronounced and still the mean remains very near that of the stock. It is possible that the crowded condition of the larvæ in the stock bottles reduced the facet number, but the real cause may be something very different.

The results of the selections in VBa indicate that there is in this race only a single hereditary factor involved in the modification of the facet number. In spite of the fact that the mean of the stock is much lower than that of either line we must assume that under the same conditions it would lie somewhere between them, in other words, if the lines could have been reared under the conditions of the stock, the low lines would have gone slightly downward due to the elimination of high numbers and the high lines would have gone slightly upward due to the elimination of low numbers. Practically pure lines were established in the first generation. This, however, may be merely apparent, the lack of further effect of selection being due to the interference of somatic factors.

The male with 34 facets, giving rise to the brood 16f1–6, must be regarded as a mutant. This conclusion is based on the great difference between this male and its brothers in 16, the definite relation between the male parent and the female offspring, and the extremely low grade of the resulting females.

In the Ba selections the results are very different and not so easily explained. Here also there must have been a downward shift as well as an upward shift. Disregarding the seventh generation for reasons already given, the following differences between the high and low lines are obtained: f1, 22; f2, 40; f3, 61; f4, 65; f5, 79; f6, 95. There is a gradual increase which becomes somewhat smaller as selection proceeds. If it were not for the interfering fluctuations the author's results would be much like those obtained by Zeleny and Mattoon. Even three additional generations of selection failed to produce pure lines.

In many respects the results obtained in these experiments resemble those obtained by MacDowell in the selection for extra bristles. The average of all the lines is raised or lowered but one can not predict within rather wide limits just what the offspring of any two parents are going to be like. This is clearly shown in Table II. and Fig. 6 in case of the matings d1f1–2 and d1f1–3. These matings were made with the same male but with females of 91 and 56 facets respectively. The average for the offspring from the higher female is considerably lower than that for the offspring of the lower female. In this case the unexpected results can not be explained on the basis that only a part of the character was observed. It is true that the counts were made only on one eye, but the difference between the two eyes falls within definite limits and is very small compared with the differences between the eyes of two flies. A comparable case would be the possibility of less than one extra bristle outside of the observed rows in the experiments of MacDowell. The large, parallel fluctuations of all the lines seem to indicate that there are environmental factors capable of almost doubling or cutting in half the facet numbers of flies of the same germinal constitution. The unexpected results must be regarded as being due to the fact that the hereditary factors are only a part of the total factor group controlling the facet number. When the environmental factors have been studied it may be possible to control them in such a way as to obtain uniform results. In that case more rigid selection will be possible.

The crosses made between the high and low lines show no evidence of sex-linkage. The offspring from both sets of matings are very nearly alike and there is no essential difference in the ratio of males and females. This agrees with the results obtained by MacDowell in his work on extra bristles. In the present experiments sex-linkage might have been expected from the fact that the ratio between male and female averages did not remain constant from generation to generation. The factors for converting the female means to the male standard in the seven generations of upward selection are successively 1.91, 1.73, 1.53, 1.40, 1.36, 1.60, 1.57. Those for the downward selections are 1.57, 1.86, 1.43, 1.79, 1.51, 1.46 and 1.40. But it is impossible to relate the differences in the offspring to differences in the parents.

The appearance of full-eyed males and heterozygous females in the stock and selected lines may be explained in two ways. It may have been due to the carelessness of the author in preparing the food, feeding and transferring the flies, or it may have been due to reverse mutation in the race.

The precautions taken by the author were not such as to exclude absolutely the possibility of the entrance of an egg here and there. But in spite of that fact the evidence is almost irrefutably against the theory of contamination. Perhaps it is well to state here in detail the author's methods. The precautions in regard to the preparation of food have already been given. It is sufficient to add that no larvæ ever appeared in the food jar. In all of these experiments the food was handled by means of an all-metal scalpel. This was used chiefly because it could be easily cleaned and could be kept absolutely clean. During the first two generations of VBa selections the author depended upon the fact that *Drosophila* does not as a rule lay eggs on a clean, dry, metal surface and merely kept the scalpel clean but did not heat it before using. In all succeeding work it was heated in an alcohol flame just before being used. All vials and bottles that had been used were boiled and rinsed in tap water and were then kept inverted on the table until they were again used. In the VBa selections no filter paper was used with the food. In the Ba selections the clean filter paper was kept in a table drawer and was removed only at the time of using. Fresh cotton for the plugs was also kept in table drawers.

If old plugs were used over again they were either sterilized and kept in closed fruit jars or they were kept in closed jars for at least a week before using. Only plugs that had not been contaminated with food were used again.

But the evidence against the theory of contamination has little to do with the precautions taken in handling the material. In the first place all flies that appeared in the vestigial-winged lines had vestigial wings and all flies that appeared in the long-winged lines had long wings. The chief reason for choosing the vestigial-winged race for the selections was the fact that the second recessive could be used as a check in case of contamination. In all of the experiments no long-winged fly appeared in the vestigial-winged lines and no vestigial-winged form appeared in the long-winged lines. If the full-eyed and heterozygous flies had been due to contamination then the other characters should also have appeared, especially since for a long time the breeding vials of the two races were intermingled and treated as one lot. In the second place the author was handling no full-eyed, vestigial-winged flies at the time the first male appeared. It is true that Professor Zeleny had his stock of such flies in the same room at the time, but they were kept on a table about twenty feet from the one used for this work and the chances that a vestigial-winged fly will travel that distance are not very great. Finally, if the flies were due to contamination, full-eyed females as well as full-eyed males should have appeared; indeed, full-eyed females should have been more frequent than heterozygous females, but all females were heterozygous.

It is interesting to note that in all cases except one where full-eyed or heterozygous flies appeared in the selected lines it was in the high lines. The one exception was the heterozygous female in h3f2, a reverse selection from a low line.

More significant, however, is the fact that the females were always heterozygous. That means that if the change took place after fertilization only one chromosome in case of the females was affected. The males, of course, have only one chromosome bearing the factor. If the change occurred before fertilization, then it is probable that it appeared in the female germ cells. Had it taken place also in the male germ cells then heterozygous females should have appeared more frequently than full-eyed males and full-eyed females might in rare instances have appeared. If the change occurred indiscriminately in male or female then the proportion of heterozygous females to full-eyed males should be 3 to 1, but the ratio obtained is 5 to 6. The numbers obtained, however, are not large enough to be conclusive.

The fact of reverse mutation is very difficult to explain. It is hard to see how the germplasm can lose a factor and still potentially retain it and have it reappear later. So far as the present data go it is possible to explain the case under consideration in two different ways.

If the normal wild fly carries a limiting factor with respect to the facet number then it is possible by partial non-disjunction for the factor to pass from one chromosome of a pair to the other, giving one chromosome without a limiting factor and the other with a double limiting factor. The bar-eyed race of *Drosophila* may be derived from such a chromosome with two limiting factors or factor groups, the mate of the chromosome having been lost in the maturation of the egg. If then in the bar-eyed race a second non-disjunction again separates the two factors the result should be one chromosome with triple factors and one with a single factor, the latter giving rise to a full-eyed male or a heterozygous female. If the former passes into the egg it should give rise to a further reduction in the facet number, but it is possible that a fly with such a chromosome does not live. It is possible also that the male with 34 facets contained a chromosome with a triple reducing factor.

A simpler explanation is that of a reversible chemical change between two compounds one of which is more stable than the other. If the compound that forms the basis for the bar eye is the less stable then reversions are to be expected under certain conditions. But the fact that the change takes place only in the female is a strong argument in favor of the theory of partial non-disjunction.

SUMMARY.

Selection was carried on in the vestigial-winged, bar-eyed stock for three generations and in the long-winged, bar-eyed stock for seven generations. The facets in the eyes of 9,000 flies were counted.

Selection in the vestigial-winged stock had to be discontinued on account of sterility and low production in the single lines.

The sterility and low production were not due to inbreeding. Sterility was not due to inability on the part of the females to lay eggs, nor does it appear to have been the final stage in the reduction of the number of offspring.

Selection in the vestigial-winged stock was effective for one generation but failed to produce further effects in the second and third generations. Return selections from the low lines were not effective.

A mutation appeared in a male in line 16. It was a reducing factor and was sex-linked. The complete sterility of the offspring of this male prevented further study of the character.

Selection in the long-winged stock was effective for six generations. In the downward selected lines most of the high flies were eliminated in the first three generations and no further effect of selection was noted. Return selections from the sixth generation, however, were still effective. The mean of the upward selected lines continued to increase at approximately uniform rate for six generations. The seventh generation must be disregarded because it was not an upward selection. The range in the facet number was not increased by selection. The results of these experiments indicate that the hereditary differences in this race of *Drosophila* are due to a large number of small factors.

Crosses made in the sixth generation between low and high lines indicate that the hereditary factors are not sex-linked.

In both the long-winged and the vestigial-winged lines the mean facet number was highly variable. This variability appeared to be due chiefly to changes in the temperature of the room, but may have been due also to other causes. The mean facet number of flies reared in vials as single lines was slightly higher than that of stock flies reared in bottles.

The mean facet number of the vestigial-winged flies was somewhat lower than that of the long-winged flies.

Six full-eyed males and five heterozygous females appeared in the stock bottles and the selected lines during these experiments. They must be regarded as reverse mutations.

The reverse mutation can be explained on the theory of a reversible chemical reaction between two compounds one of which is more stable than the other. The present facts, however, favor an explanation on the basis of partial non-disjunction.

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MITOSIS AND AMITOSIS.

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It was once supposed that all forms of nuclear division were of the type which is now called amitosis in which the nucleus undergoes simple constriction. After the discovery of the complicated method of nuclear division now known as mitosis and the recognition of its very general occurrence in plants and animals, the doubt was expressed whether amitosis ever occurs as a normal process; its occurrence in pathological and degenerating cells is undoubted. However, the number of cases in which nuclear constriction is known to occur regularly in certain tissue cells is too great to warrant the belief that it is always a pathological phenomenon.

The only question of present importance in connection with this form of nuclear division is as to its bearing on the chromosome theory of heredity. If amitotic division of the nucleus, followed by division of the cell body, ever occurs in the germ cell cycle or in cleavage cells undergoing normal differentiation it would go far to disprove the "individuality" or persistent identity of chromosomes and consequently the chromosome theory of inheritance, since it is scarcely possible that individual chromosomes can be equally divided and their halves accurately distributed to daughter cells by the process of amitosis. The majority of cytologists at the present day concur in the opinion that amitosis does not normally occur in embryonic or germ cells and that when it does occur in such cells the ensuing development is abnormal. On the other hand some investigators maintain that amitosis occurs regularly and predominantly in the genesis of the germ cells and also in the blastomeres and embryonic cells of several species of animals and plants and for this reason among others they reject the chromosome theory. Child in particular has maintained this position in a series of papers (1907–1911) dealing primarily with the cestode *Monezia*, but secondarily with several other animal forms ranging all the way from cœlenterates to birds.

Among other recent writers who have maintained a similar view are Hargitt (1904, 1911) on amitosis in the cleavage of the eggs of cœlenterates, Patterson (1908) in blastoderm cells of the pigeon, Glaser (1908) in embryonic cells of *Fasciolaria*, Jordan (1908) in spermatogonial divisions of *Aplopus*, Nathansohn (1900) in *Spirogyra*, Wasielewski (1902, 1903) in the root tips of *Vicia*, Gurwitsch (1905) in the blastomeres of *Triton*, Maximow (1908) in embryonic tissues of the rabbit, Jörgensen (1908) in the oögenesis of *Nephelis*, Moroff (1909) in the eggs of copepods, Knoche (1910) in the insect ovary, Nowikoff (1910) in bone and sinew cells of the young mouse, and Foot and Strobell (1911) in the ovary of *Protenor*—all of whom hold that amitosis may occur as a normal process in germinal and embryonic cells. Several of the authors named as well as R. Hertwig (1898), Lang (1901), Calkins (1901), Herbst (1909), Godlewski (1909) and Konopacki (1911) hold that there is no principal distinction between mitosis and amitosis and that they may both occur without interfering with normal processes of differentiation.

On the other hand, this view is contested by Boveri (1907) and Strasburger (1908) on general grounds and is not confirmed by the experiments of Häcker (1900) and Schiller (1909) on *Cyclops* eggs subjected to ether, nor by the experiments of Němec (1903) who repeated the work of Wasielewski on the root tips of *Vicia* subjected to chloral hydrate and reached the conclusion that the supposed amitoses are really modified mitoses. Richards (1909, 1911) and Harmon (1913) carefully and laboriously repeated Child's work on cestodes and found no evidence of amitosis in germ cells or cleavage cells, while on the other hand there was abundant evidence of mitosis. Child (1911) has reëxamined the question and reaffirms in the main his former opinion, and this has been confirmed by Young (1913), whose general conclusions, however, are so reactionary and even revolutionary that they cannot be accepted without further confirmation.

Boveri (1907) has said that if Child would establish his contention he must prove (1) that the binucleate condition which he finds is actually due to a division, (2) that a portion of the cytoplasm is cut off around each of these nuclei, (3) that the cells arising in this manner again divide mitotically and have the normal number of chromosomes; in his opinion not one of these proofs has been furnished. The binucleate or multinucleate condition, he adds, may be due to an incomplete fusion of chromosomal vesicles after mitosis, as Rubaschkin (1905) has shown to be true for the blastomeres of *Triton*. Boveri concludes, therefore, that the individuality theory has not been shaken by this work on amitosis. There is no doubt that constricted or lobulated nuclei are sometimes found in germ cells or embryonic cells; the critical question is whether these are stages in the amitotic division of the nucleus and if so whether the cell bodies divide and are capable of normal development. It is evident from a large number of observations on a great variety of objects that constricted nuclei are not in themselves sufficient evidence that amitosis is occurring, for many such nuclei of living cells have been observed to lose the constriction and later to divide by mitosis. And even if such constricted nuclei actually divide, the chromosomal constitution of the cell could remain normal provided the cell body did not divide following the amitotic division of the nucleus.

As a matter of fact, amitotic division of the nucleus is rarely followed by division of the cell body. Macklin (1916) has recently studied amitosis in living tissue cells of the embryo chick and, although he was able to follow the process of nuclear division in successive stages in one and the same cell, in no case was such amitotic division of the nucleus followed by division of the cell body. Such amitotic division was sometimes followed by mitosis and in such cases the two or more nuclear vesicles in a cell gave rise to chromosomes which formed a single equatorial plate. Macklin concludes, therefore, that "there is nothing in nuclear amitosis opposed to the chromosome hypothesis"—with which opinion I entirely agree.

Unfortunately it is rarely possible to study all stages of nuclear and cell division in normal living cells, not only because of the indistinctness with which one sees what is going on inside the cell, but also because of the difficulty of keeping cells alive and normal for a sufficient length of time under conditions of observation; furthermore, failure to observe amitosis in one hundred such cells would still leave it possible that it might occur in a second hundred. There is, however, a method by which the occurrence or non-occurrence of amitosis can be determined with great certainty and this is in the study of identical cell divisions in hundreds and thousands of individuals. This is possible only in the two maturation divisions which are so peculiar that they can always be distinguished from each other and from all other divisions, and in those early cleavages where the lineage of every cell is known and its method of division can be observed in thousands of different cases. If in every such dividing cell one sees mitosis only, it can be concluded that this is the invariable method of division in these cells. It is a significant fact, which Child himself admits, that in no such case have the nucleus and cell ever been seen to divide by amitosis, whereas in hundreds and thousands of cases they have been seen to divide by mitosis.

Nevertheless, in these very cells one frequently observes lobulated, constricted or bipartite nuclei which might be regarded as stages in amitosis were it not for the fact that the study of the lineage of these cells shows that they invariably divide by mitosis and that the peculiar shapes of the nuclei referred to are due to modifications of normal mitosis. Such constricted or bipartite nuclei occurring in cells the lineage of which was unknown would naturally be mistaken for cases of amitosis and it is very significant that the reports of amitosis in embryonic cells have been invariably in cells of unknown lineage.

This paper is based on a study of modified mitosis in the maturation and cleavage of the eggs of the marine gasteropod, *Crepidula plana*. A variety of nuclear forms are figured and described which resemble more or less closely stages of amitosis and yet it is perfectly certain that these nuclei divide only by mitosis, and the manner of origin of these pseudo-amitotic forms is plainly due to modifications of regular mitotic processes. These modifications were produced by subjecting dividing cells to abnormally high temperatures or to sea-water of abnormal densities, but other similar modifications are produced by many other abnormal conditions such as pressure, centrifugal force, carbonic acid and various other chemical substances (Conklin, 1912). These different types of modified mitoses may be classified under the following heads: (1) Scattering of chromosomes and their failure to unite into a single nuclear vesicle, (2) amitotic or mitotic division of the nucleus without division of the cell body and the subsequent division of such binuclear or polynuclear cells, (3) separation of chromatin and achromatin and formation of cytasters, (4) persistence of nuclear membrane and formation of chromatic connections between daughter nuclei.

I. Scattering of Chromosomes and Their Failure to Unite Into a Single Nuclear Vesicle.

In the late anaphase of normal mitoses the chromosomes of the daughter plate stick together so that when the individual chromosomes begin to take in achromatic substance and to swell up into chromosomal vesicles the whole plate is converted into a mulberry-like mass which later becomes a single nuclear vesicle either by the fusion of the separate chromosomal vesicles or by their closer approximation. There is a growing body of evidence that in certain cases at least these closely appressed chromosomal vesicles do not completely fuse with one another but preserve their individuality (Bonnevie, 1908, for *Ascaris* and *Allium*; Vejdovsky, 1912, for *Ascaris* and *Decticus*; Wenrich, 1916, for *Phrynotettix*; Richards, 1917, for *Fundulus*). In other cases, when it is not possible to recognize a distinct vesicle for each and every chromosome, maternal and paternal chromosomes may form more or less distinct vesicles (Häcker, 1895, for *Cyclops*; Conklin, 1901, 1902, for *Crepidula*).

In certain abnormal conditions, and especially by means of temperatures higher than normal and by hypertonic sea-water, the division and separation of daughter chromosomes may be delayed or stopped and the chromosomes scattered along the length of the spindle (Figs. 9, 10, 29). After the chromosomes have reached the poles of the spindle, they may be separated from one another and remain scattered more or less widely in the cell. If the temperature is not too high (34°–35° C.) each separate chromosome will then swell up to form a separate vesicle, or if two or more chromosomes are in close contact they may form a single vesicle of larger size (Figs. 7, 9–12).

If the temperature is a little higher (37° C.) the chromosomes may stick together in an irregular mass and be drawn to the surface of the cell, probably by the complete contraction of the fibers which anchor the spindle to the peripheral layer of the protoplasm (Figs. 13, 14, 17). In the latter instance the chromosomes do not swell up and become vesicles, but remain permanently small and densely chromatic. Owing to some change, probably in the peripheral layer of each chromosome, caused by the high temperature, the chromosomes are unable to take up achromatin. Such chromosomes and the cells containing them never recover and never go further in development, although the cells do not immediately undergo degeneration and to all appearances remain alive for twenty-four hours or longer.

Of a piece with this scattering of chromosomes is the failure of all the chromosomal vesicles to unite into a single vesicle. All degrees of fusion of chromosomal vesicles may be found from those which remain wholly separate to those which are united into a single spherical vesicle. Other things being equal, the size of a nuclear vesicle varies according to the number of chromosomal vesicles which enter into it. In this way arise nuclear forms which have been called "fragmented nuclei," "multinuclear cells," "bipartite," "lobulated" and "elongated" nuclei (Figs. 1–12, 19–28), although it is evident in the case of *Crepidula* that these nuclear forms have not arisen by constriction or fragmentation of an originally single nucleus.

These partial nuclei formed by incomplete fusion of the chromosomal vesicles were first called "karyomeres" by Fol. That they are partial and not entire nuclei is shown by the fact first established by Boveri that in the next following mitosis each karyomere gives rise only to the same number of chromosomes as entered into it and not to the full number of chromosomes characteristic of the species; this fact I have repeatedly confirmed in my studies on *Crepidula*. And that such karyomeres are due to the failure of chromosomes to unite into one vesicle and not to the amitotic division of a single vesicle is shown by the following considerations:

1. They are most numerous in the telophase of division (Figs. 7–12) where the chromosomes are sometimes widely scattered

and where every chromosome may give rise to a separate chromosomal vesicle (Fig. 7). As the cell passes into the resting phase, these vesicles fuse together more or less completely, giving rise to vesicles of varying sizes. Other things being equal, the later the stage in the resting period the smaller the number of separate vesicles. If the separate vesicles were formed by division of an originally single vesicle, exactly the reverse would be the case.

2. The presence of centrospheres and spindle remnants in many cases shows conclusively that division has taken place by mitosis, and the position of these structures indicates the location of the mitotic figure (Figs. 8–12 *et seq.*).

3. The elongation of daughter nuclei in the position of the plate of daughter chromosomes of normal division figures indicates that such elongated nuclei are formed by the partial fusion of the chromosomes of the daughter plate (Figs. 4–6).

These considerations make it absolutely certain that these peculiar nuclear forms are due to a partial or incomplete union of chromosomes into a single nuclear vesicle in the final phase of mitosis; they represent modified mitosis and not amitosis. And it is practically certain that many of the cases of so-called amitosis described by several of the authors mentioned above are of this same character.

It is not easy to determine exactly the mechanism by which these modifications have been produced. As already mentioned they may be caused by abnormally high temperature (Figs. 1–12) or by hypertonic sea-water (Figs. 19–29). It is well known that when the daughter chromosomes approach the poles of the spindle they are normally closely crowded together and it seems probable that this is due to linin connections within the spindle or between the chromosomes. In the development of the daughter nucleus each chromosome absorbs achromatic material from the cytoplasm and becomes a vesicle with chromatic walls. The material thus absorbed is probably chiefly water though it doubtless contains dialyzable proteins and other substances which may be assimilated into the chromatin and linin of the nucleus. It is probable that there is a real membrane surrounding each chromosome (Conklin, 1902, 1912) and that the absorption of surrounding substances by the chromosome takes place through this membrane by a process of dialysis. The nuclear vesicle is most nearly spherical in form when it is largest and when its contents are most fluid in character irregular or lobulated nuclei are usually smaller and the nuclear contents more dense (Figs. 1–6). Therefore the modifications of mitosis, which prevent the union of chromosomes into a single vesicle, act by modifying the walls of these vesicles so that they do not readily unite and so that they do not readily absorb fluid from the cytoplasm.

II. Amitotic or Mitotic Division of Nuclei Without Division of Cell Body and Subsequent Division of Such Binuclear or Polynuclear Cells.

In the cleavage of *Crepidula* a binucleate or multinucleate cell is invariably due, so far as I have observed, to a failure of chromosomal vesicles to unite into a single vesicle. In other cases, however, it is plain that elongated, constricted and bipartite nuclei have resulted from the constriction of a single nuclear vesicle. In addition to numerous cases which have been described by other investigators, I have myself studied such cases in the egg-follicle cells of *Gryllus* (Conklin, 1903), the liver cells of *Porcellio* (Conklin, 1897), as well as in some muscle cells and connective tissue cells. In none of these cases, however, is division of the nucleus followed by division of the cell body. Although every follicle cell of *Gryllus* and every liver cell of *Porcellio* shows the nucleus in some stage of amitosis, and although many of these cells contain two entirely separate nuclei, in no single instance have I ever seen a division of the cell body separating these nuclear halves.

Since these cases of amitosis occur in differentiated tissue cells it may be assumed that the nuclei are not active in the further differentiation of these cells; on the other hand their metabolic activity is great and the nuclei are undoubtedly concerned in this activity. It has been assumed that the division or lobulation of such nuclei favors metabolic activity by increasing the surface of the nuclei and bringing them into closer relation to all of the cytoplasm of the cell, and this is probably true. Especially in the case of elongated muscle cells the division of the nucleus and the distribution of the daughter nuclei along the length of the fiber must facilitate interaction between nucleus and cytoplasm, and the same is true, though perhaps to a smaller extent, in gland cells and egg-follicle cells.

Dahlgren and Kepner (1908) hold that the very numerous amitoses in the striated muscle cells of the embryo fish, *Catostomus*, may be followed in some instances by the division of the muscle cell. But since the plane of nuclear division is always transverse to the fiber, while the plane of cell division is always longitudinal, it could not be affirmed that the cell divisions in this case correspond to the nuclear divisions. But even if amitoses may be followed by division of the cell body in these cases, it must not be forgotten that all these cells are fully differentiated and according to the chromosome theory the nucleus has already performed its differentiating functions while its further function in the fully differentiated cell is probably purely trophic.

Many observations and experiments demonstrate that the nucleus is concerned in the two functions (1) of differentiation or regulation and (2) of metabolism; the work of Gruber (1886), R. Hertwig (1898), Heidenhain (1894), Henneguy (1896), Conklin (1902) *et al.* indicates that the chromosomes or basichromatin are particularly concerned with the former, the oxychromatin or achromatin with the latter. It is a significant fact that chromosomes divide only by mitosis and Boveri (1908) has shown that a complete set of chromosomes is necessary to normal differentiation. On the other hand oxychromatin and achromatin divide only by amitosis even in cases of mitotic division of the chromosomes. The significance of these facts seems to have been missed not only by those who maintain the equivalence of mitosis and amitosis, but also by Weismann and his followers who assumed that in embryonic differentiation there is a differential division of chromosomes and a "disintegration of the germ plasm" with segregation of particular factors in particular cells. For if individual chromosomes differ in hereditary potencies, every division by amitosis must be a differential one, while on the other hand every typical mitotic division is non-differential so far as the chromosomes are concerned.

Since it is usually impossible to see outlines of individual chromosomal vesicles in the resting nucleus, it is not possible to determine whether the constrictions and lobulations of amitosis merely separate whole vesicles from one another. If they do the number of chromosomes arising from each of these vesicles in subsequent mitosis should be the same as in the case of karyomeres formed by the failure of vesicles to unite in the resting stage.

It is probable from the work of Boveri (1907) and of Macklin (1916) that when amitotic division of the nucleus is followed by mitosis, each nuclear vesicle gives rise to a fraction only of the normal number of chromosomes and that all the nuclear vesicles in a cell taken together give rise to no more than the normal number. Furthermore, the work of Boveri demonstrates that there is no return to the normal number of chromosomes when once a cell contains an abnormal number. Each nuclear vesicle produced by amitotic division is therefore a karyomere, in every way comparable to those produced by incomplete fusion of chromosomal vesicles; it is a fragment of a nucleus and not an entire nucleus, and this is equally true whether all the karyomeres lie within a single cell, as is usually the case, or whether in some rare instances they may be distributed to separate cells. In *Crepidula* it matters not how many karyomeres there are in a cell, if there are two and only two centrosomes all the chromosomes come together into a single plate and there is a normal division and distribution of each of these chromosomes to the daughter cells.

Therefore in considering the significance of amitosis it is of the utmost importance to know whether the constriction of the nucleus is followed by a division of the cell body; if it is not, amitosis is not a permanent nuclear division at all but merely a temporary separation of karyomeres which come together again into a unit structure at the next mitosis. It is a significant fact that in most instances amitotic division of the nucleus is not followed by division of the cell body.

In this connection it is worth while to compare with the conditions just described those which obtain when mitosis is not followed by division of the cell-body. In a former paper (Conklin, 1912) I have described such cases at some length and need not here go into details. In brief, if the daughter nuclei and centrosomes lie so far apart in the undivided cell body that they do not interfere, in subsequent mitoses every one of these mitoses may be entirely normal and development may be typical except that no differentiation ever appears between the halves of the undivided cell. On the other hand, if the daughter centrosomes lie near together in the undivided cell body so that they interfere we get tripolar or tetrapolar figures with irregular distribution of chromosomes and usually with the formation of several karyomeres of varying sizes depending upon the number of chromosomes entering into them. Such multipolar mitoses in *Crepidula* are rarely followed by division of the cell body so that at every succeeding mitotic period the number of centrosomes and chromosomes in this undivided cell body are approximately doubled (Figs. 26–29). Of course such cells with abnormal numbers of chromosomes and centrosomes never develop normally. Normal differentiation depends upon the regular distribution into separate cells of daughter centrosomes and chromosomes as well as of different cytoplasmic substances.

III. SEPARATION OF CHROMATIN AND ACHROMATIN AND FORMATION OF CYTASTERS.

The behavior of the chromatic and achromatic parts of the nucleus in hypertonic and in hypotonic media throws a certain amount of light on the constitution of the normal nucleus and on the behavior of these nuclear constituents during normal mitosis. When resting nuclei are subjected to hypertonic solutions (*e. g.*, 2–4 per cent. NaCl in sea-water) the chromatic portion of the nucleus contracts into a small dense mass leaving the achromatic portion as large as ever (Figs. 32, 43, 48). It looks as if the chromatin had undergone complete "plasmolysis" whereas the volume of the achromatin had not been affected at all. The membrane or boundary of this achromatin remains full and unshrunken, which would presumably not be the case if this outline represented a real plasma membrane. The shrinkage of the chromatin on the other hand probably indicates that it is surrounded by a plasma membrane, or more likely that each chromosome is so surrounded.

When resting nuclei are subjected to hypotonic solutions the entire nucleus becomes slightly swollen and less deeply chromatic, which indicates that the chromatic parts of the nuclei take up water, probably through the chromatic nuclear membrane or the chromosomal membranes.

The achromatic membrane, or rather boundary, is regularly spherical in resting stages but during mitosis it disappears or else becomes so indefinite and irregular in outline that it is difficult to recognize. However the achromatic substance of the nucleus together with some of the denser portion of the cytoplasm constitutes the amphiaster with its nuclear spindle and astral radiations. In hypertonic solutions the amphiaster is sharply set off from the surrounding cytoplasm (Figs. 35–36), due as I believe in the main to the condensation of its substance and the elimination from it of the more fluid cytoplasm. In this process of condensation the astral radiations are largely drawn into the central part of the figure but portions of these radiations may become isolated from the amphiaster and thus form independent condensation centers. These have a radiating structure and are typical cytasters, but unlike those described by Wilson they do not in *Crepidula* divide nor form the poles of true mitotic figures. My observations on the origin and nature of these cytasters (Conklin, 1912) entirely agree with those of Konopacki (1911) and in the main with the observations of Mead (1898) and Morgan (1899).

Cytasters appear best developed during periods of mitosis when the achromatin is distributed in the astral radiations (Figs. 33–35) but they are also abundant in eggs after the maturation divisions and before the first cleavage (Figs. 31, 32) and in such cases one can frequently see that they lie along the radiations of the maturation aster (Fig. 31).

During prolonged resting periods, especially when the eggs are in strong salt solutions, cytasters are replaced by faintly staining vesicles (Figs. 37–42) which appear to contain achromatic nuclear material. These vesicles are surrounded by a delicate achromatic membrane and they resemble R. Hertwig's "nuclei without chromatin." They are found chiefly in the position of the previous spindle remnants and along the lines of astral radiations. Usually the largest of these achromatic vesicles are in close proximity to the dense mass of chromatin, which in these cases does not become vesiculated. In some instances there is a single elongated achromatic vesicle in each daughter cell which occupies the position of the interzonal fibers of the spindle (Figs. 43–48) and which may inclose the dense mass of chromosomes at the spindle ends. Such conditions give the appearance of an amitotic division of the nuclear vesicle, but the presence of centrosomes, mid-bodies and in some instances of spindle fibers and astral radiations as well as of chromosomal plates (Figs. 46, 47) clearly shows that these divisions are true mitoses in which the chromosomes have been prevented from absorbing achromatin while the latter has formed a definite boundary or membrane separating it from the cytoplasm.

Just as the size of a central aster is reduced by the presence of numerous cytasters or parasitic asters which surround it, so the size of the chromatic nuclear vesicle is inversely proportional to the volume of the achromatic vesicles in the cell. It seems practically certain that the chromosomal vesicles and consequently the entire chromatic portion of the nucleus grow by the absorption of this achromatic substance. When nuclei are large they contain much achromatic substance and at the same time there are no cytasters or achromatic vesicles in their vicinity; when they are small and densely chromatic there may be cytasters in the cell during the periods of mitosis, or achromatic vesicles during resting periods. "The cytasters are therefore, in my opinion, isolated portions of archiplasm (achromatin plus spongioplasm) derived in large part from escaped achromatin, which take the aster form during mitosis and the vesicular form during resting periods" (Conklin, 1912, p. 543).

IV. Persistence of Nuclear Membranes and Formation of Chromatic Connections Between Daughter Nuclei.

It has generally been assumed that one of the strongest evidences that amitosis had occurred in any given case was to be found in the incomplete separation of daughter nuclei or in chromatic connections between them. Thus Gurwitsch (1905) has figured and described the division of a blastomere of a centrifuged *Triton* egg in which two nuclei, connected by a chromatic thread, are dividing by mitosis. The chromatic connection is taken as proof positive that the nuclei had divided by amitosis and Godlewski (1909) in a general review of this subject, after dismissing as doubtful many other cases in which amitosis had been reported as occurring in normal development, falls back upon this case described by Gurwitsch as one of the strongest evidences in favor of the view that amitosis may occur in normally differentiating cells.

But chromatic connections between nuclei are not to be taken as positive evidence that those nuclei have divided by amitosis, for these connections may be the result of incomplete or atypical mitoses. Anything which retards or prevents the separation of daughter chromosomes may lead to the scattering of chromosomes along the spindle or to their elongation into threads and consequently to the formation of chromatic connections between daughter nuclei. Häcker found such connections in etherized eggs of *Cyclops* and such connections are present also in *Crepidula* eggs subjected to high temperatures (Figs. 6, 8), to hypertonic sea-water (Fig. 29), and to hypotonic sea-water (Figs. 49–60). It is especially in the last named experiments that chromatic connections between daughter nuclei are most frequently seen and they merit a detailed description.

The eggs shown in Figs. 49–54 were placed for one hour in sea-water diluted with two volumes of fresh water and were then returned to normal sea-water for four hours before being fixed; those shown in Figs. 55–60 were placed for two hours in sea-water diluted with one volume of fresh water and were then left in normal sea-water for fourteen hours. In all of these cases the centrosomes divided normally and approximately normal spindles were formed but the separation of chromosomes and the formation of daughter nuclei were atypical. In Figs. 9, 29, 49 and 50 the scattering of chromosomes is shown in some of the spindles but more notable than this is the stretching of chromosomes into long threads some of which run from one pole of the spindle to the other. When chromosomes are merely scattered throughout the cell or along the spindle they usually give rise to chromosomal vesicles wherever they lie, as is shown in Figs. 7, 16, 26, etc., but when in addition they are stretched into elongated threads chromatic connections are left between daughter nuclei.

In diluted sea-water the chromosomes show a tendency to stick together into masses and to stretch out into long threads instead of dividing and moving to the two poles of the spindle. This is probably due to the fact that the linin basis of the chromosomes is modified so that the latter do not preserve their usual shapes and do not separate normally in division. The nuclear membrane also frequently remains chromatic and in such cases may persist throughout mitosis (Figs. 49–53, 55, 59, etc.). Evidently some of the chromatin which usually enters into the formation of the chromosomes is left in these cases at the periphery of the nucleus. Since the nucleus is composed of chromosomal vesicles more or less completely united this result might conceivably be due to the swelling and bursting of some of these vesicles at the nuclear periphery.

When mitosis is halted in the prophase of the third cleavage the centrosomes separate and a spindle is formed in the usual manner but the nuclear membrane persists and the entire nucleus becomes pear-shaped (Fig. 49, cell D), or unequally constricted (Figs. 51, 52), the smaller portion corresponding to the micromeres containing almost all of the deeply staining chromatin in the form of chromatic threads, while the larger portion belonging to the macromeres contains faintly staining threads and granules. These two portions of the constricted nuclei are approximately proportional in size to the nuclei of the normal micromeres and macromeres, although in cell D, Fig. 49, and cells C and D, Fig. 52, the division wall between the micromeres and macromeres has not formed. The fact that these two portions of the constricted nuclei are proportional in size to the cells to which they belong even when the division wall between those cells has not formed is difficult to explain. Generally the size of a nucleus is proportional to the volume of cytoplasm in which it lies (Conklin, 1912) because the chromosomal vesicles absorb substance from the cytoplasm in their growth, but in this case the entire nucleus has undergone constriction and the cell body has not.

The aggregation of chromatin on the side of the nuclear vesicle on which the spindle lies causes it to collect on the animal pole side of the nucleus in Figs. 49–51 and on the side away from the animal pole in cell D, Fig. 54. In every instance the chromatin collects at that pole of the nucleus which is next to the centrosome or spindle; this is a general phenomenon which has been remarked by R. Hertwig in *Actinospherium*, by Calkins in *Noctiluca*, Conklin in *Crepidula*, etc. In this connection one recalls that in many protozoa the nuclear membrane persists throughout mitosis the spindle being within the nuclear vesicle. The chromosomes divide and separate as in typical mitosis but the nuclear membrane and vesicle constrict as in amitosis. In the ciliata the micronucleus divides by mitosis, the macronucleus by amitosis. In metazoa also the chromosomes alone divide by mitosis, or the splitting of the chromatic thread, while the division of all other nuclear constituents is a mass division.

The peculiar form of nuclear division which is caused by the stretching out of the chromosomes and the persistence of the nuclear membrane superficially resembles amitosis but is really a modified form of mitosis. Nuclei which have divided in this peculiar manner go on dividing by mitosis when they are returned to normal sea-water. Thus cells A and C, Figs. 49 and 50, cells A and B, Figs. 52 and 53, and all the macromeres in Fig. 54 are shown dividing by mitosis. These are typical mitotic figures though the number and arrangement of chromosomes may be atypical. In all of these cases the dividing nucleus of the macromere is connected with the nucleus of the micromere by a chromatic strand and it is particularly noticeable that this strand always runs to the plate of chromosomes in the macromeres and usually to the outer side of this plate. Since the original chromatic connection united the nuclei by their distal poles (away from the centrosome) the fact that when these nuclei divide the connection runs to the proximal pole (toward the centrosome) indicates that the chromatin changes position within the nuclear vesicle, being drawn to the proximal pole of the nucleus (Fig. 54, *D*).

Again the way in which the chromatic connection between nuclei unites with the chromosome plate (Figs. 53, 54) shows that this connection is actually composed of chromosomal substance though it has been so modified that it does not give rise to separate chromosomes nor does it show any tendency to divide or split, as normal chromosomes do, into daughter chromosomes. Although it is not possible to count the number of chromosomes in these plates it is evident that it varies in different cases and that in general it is less than normal, which is what would be expected if the chromatic connections represent a number of spun-out chromosomes. Furthermore the fact that these connections do not swell up to form vesicles as normal chromosomes do, indicates that the chromosomal substance of which it is composed has undergone some significant change.

Still later divisions of the cells connected by these chromatic strands are generally abnormal, as is shown in Figs. 55–60. The eggs shown in these figures were subjected to diluted sea-water during the third cleavage and were then returned to normal sea-water where some of the cells have undergone the fourth, fifth and sixth cleavages. In many cases the chromosomes at each of these cleavages have been stretched out into chromatic connections between daughter nuclei and since all of these persist a complicated network of such connections is present between nuclei of successive generations (Figs. 55, 56, 58, 60); at the same time the number of chromosomes in a plate is in many instances greatly reduced (Fig. 55).

The same types of modifications produced by diluted sea-water persist after the eggs have been returned to normal sea-water and in all of these cleavages, if one saw only the end result, the chromatic connections would seem to indicate that the nuclei had divided by amitosis. However a study of various stages in this process shows conclusively that this is not the case but that all of these divisions are modified forms of mitosis.

V. Conclusions.

1. The modern revival of interest in amitosis is due to a reaction against the chromosome theory. If nuclear and cell divisions ever take place by amitosis in normally developing sex

cells and embryonic cells it would deal a fatal blow to that theory. The occurrence of amitosis in fully differentiated tissue cells or in cells which do not undergo division would not affect the chromosome theory.

2. When direct division of the nucleus occurs it is rarely if ever accompanied by division of the cell body. The individual nuclear vesicles or karyomeres are not whole nuclei but fragments of a nucleus and when the cell actually divides these karyomeres combined form the typical number of chromosomes which unite into a single spindle and divide in the typical manner, as recently shown by Macklin.

3. Many apparent cases of amitosis are merely modified mitoses of which the following forms are described in this paper:

(*a*) The scattering of chromosomes and their failure to unite into a single nuclear vesicle.

(*b*) Mitotic division of the nucleus without division of the cell body and the consequent formation of binucleate or polynucleate cell.

(*c*) The failure of daughter chromosomes to pull apart in the spindle and the consequent formation of chromatic connections between daughter nuclei.

(*d*) The persistence of the nuclear membrane with division of the chromosomes by mitosis and of the nuclear vesicle by constriction.

4. There is not a single wholly conclusive case in which amitosis has been shown to occur in the division of normally differentiating cells. Therefore the attempts to disprove the chromosome theory in this way have failed.

5. Mitosis and amitosis are fundamentally unlike. Mitosis is the one and only method of bringing about equal division and distribution of the chromatic material of the nucleus. Amitosis is not a genuine divisional phenomenon at all but merely a means of increasing the nuclear surface and of distributing nuclear material throughout the cell, comparable to nuclear lobulation, fragmentation or distribution. These two processes are not equivalent or even comparable nor may one of them be converted into the other.

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DESCRIPTION OF FIGURES.

FIGS. 1–60 represent whole eggs of *Crepidula plana* which had been fixed, stained and mounted. All figures were drawn with camera lucida under Zeiss apochromatic objective 3 mm. Hom. Im., Comps. ocular 4. As drawn they represent a magnification of 333 diameters; in the process of reproduction they have been reduced about one third.

FIGS. 1–18 represent eggs which had been subjected to increased temperature.

Explanation of Plate I.

Fig. 1. No. 1174(2). 37°, ¼ hr., during second maturation; then kept at room temperature (ca. 27°) for 3 hrs. The first polar body is shaded by transverse lines; the sperm nucleus is shown at the left; the other nuclei are karyomeres formed by the scattering of chromosomes of the second maturation mitosis.

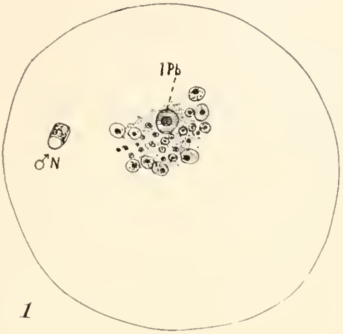
Fig. 2. No. 1175(2). 33°, ¼ hr., during first cleavage; room temperature (ca. 27°) 3 hrs. The imperfect fusion of the karyomeres (gonomeres?) gives an appearance of amitosis to each of the nuclei.

Fig. 3. No. 1176(2). 34°–35°, ¼ hr., during second cleavage; room temperature (ca. 23°) 3 hrs. Karyomeres formed by scattering of chromosomes of second cleavage.

Fig. 4. No. 1173(2). 37°, ¼ hr., beginning of third cleavage; room temperature (ca. 27°) 3 hrs. Many karyomeres formed by scattering of chromosomes of third cleavage. Traces of centrospheres are shown in the cells.

Fig. 5. No. 1176(2). 34°–35°, ¼ hr., during third cleavage; room temperature (ca. 23°) 3 hrs. Karyomeres formed by scattering of chromosomes of third cleavage. In one quadrant a micromere was formed, in the other quadrants cell division was suppressed. Traces of centrospheres as in preceding.

Fig. 6. No. 1173(2). 37°, ¼ hr., during anaphase of third cleavage; room temperature (ca. 27°) 3 hrs. In two quadrants micromeres have formed. Daughter nuclei are elongated in the greater dimensions of the chromosome plates, the latter having fused together into irregular masses.



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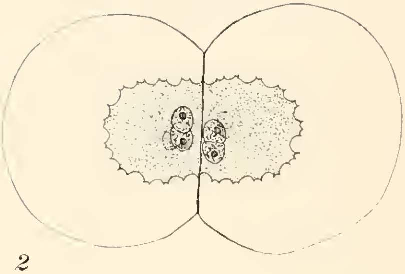

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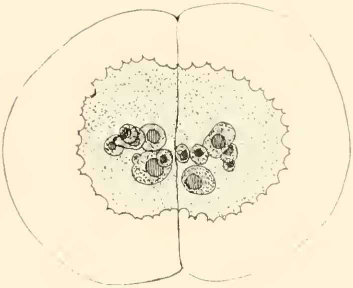

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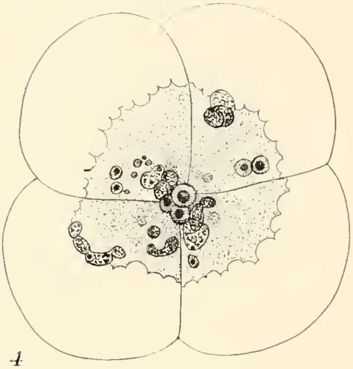

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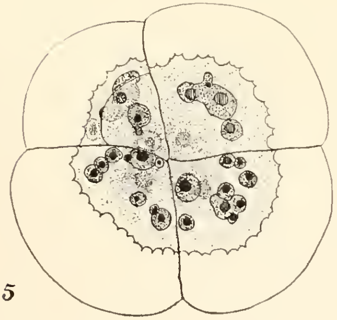

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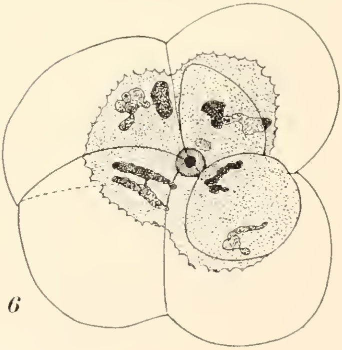

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Explanation of Plate II.

Fig. 7. No. 960. Ca. 35°, 4 hrs., during third cleavage. The chromosomes have scattered and formed numerous chromosomal vesicles.

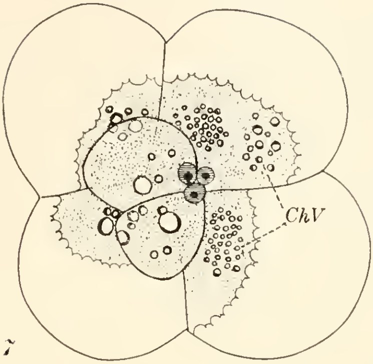
Fig. 8. No. 1176(2). 34°–35°, $\frac{1}{4}$ hr., during anaphase of third cleavage; room temperature (ca. 23°) 3 hrs. Many nuclei are lobed or irregular; nuclei are most abnormal in the quadrant in which a micromere did not form, and in which mitosis was not so far advanced at the time of the experiment, as in the other quadrants.

Fig. 9. No. 1176(1). 34°–35°, $\frac{1}{4}$ hr., during fourth cleavage of macromeres. In three quadrants the chromosomes are scattered in the spindles, in the fourth are chromosomal vesicles. The nuclei and centrosomes in the first set of micromeres are in a resting stage and are absolutely normal.

Fig. 10. No. 1176(1). 34°–35°, $\frac{1}{4}$ hr., during fourth cleavage. Chromosomal vesicles are present in one of the macromeres, and chromosomes are more or less scattered in all micromeres of the first set.

Fig. 11. No. 1176(2). 34°–35°, $\frac{1}{4}$ hr., during fourth cleavage; room temperature (23°) 3 hrs. The nuclei formed after the division of the first set of micromeres are lobed and irregular; the scattered chromosomes in the macromeres have formed many karyomeres. This figure shows an egg like Fig. 9 after being kept three hours at normal temperature.

Fig. 12. Same slide as preceding. Many lobulated nuclei and karyomeres in the cells derived from the first set of micromeres; nuclei in second set of micromeres and in macromeres are nearly normal. This figure shows an egg like Fig. 10, after being kept three hours at normal temperature.



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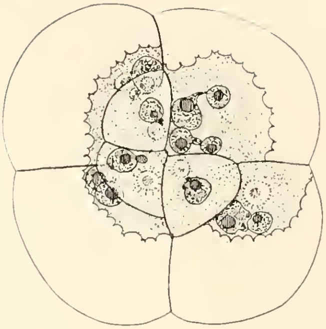

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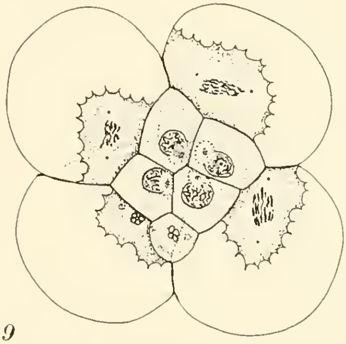

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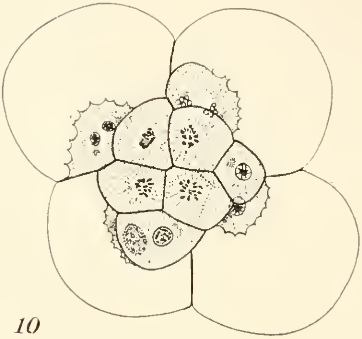

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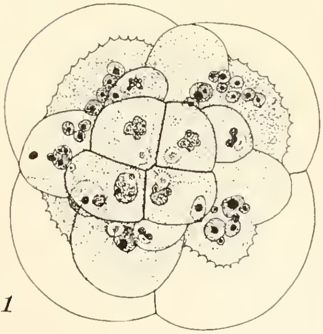

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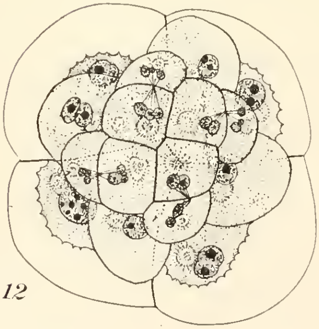

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Explanation of Plate III.

Figs. 13–18 were subjected to temperature so high (37°), or for so long a period (35° for four hours) that further cell division was stopped in almost all cases, though the protoplasm remained transparent and apparently alive.

Fig. 13. No. 1171(2). 37°, ½ hr. during first cleavage; room temperature (ca. 25°) 15 hrs. Astral areas are large and distinct; chromosomes are widely scattered or clumped in two principal masses outside the astral areas and at the surface of the egg; chromosomes do not become vesicular.

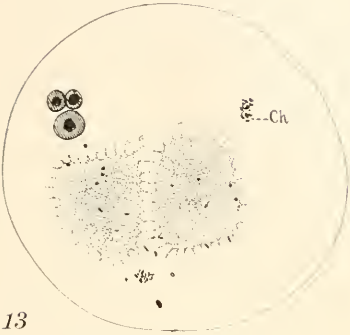
Fig. 14. Same slide as preceding;·subjected to heat during second cleavage, and showing results similar to preceding.

Fig. 15. No. 960. Ca. 35°, 4 hrs., during resting 2-cell stage. The plasma is much contracted and vacuolated and the chromatin of the nuclei is in the form of hollow spheres, which look like chromosomal vesicles.

Fig. 16. Same slide as preceding; heated during second cleavage mitosis; plasma shrunken and chromosomes in the form of vesicles.

Fig. 17. No. 1171(2). 37°, ½ hr., during fourth cleavage; room temperature (ca. 25°) 15 hrs. Chromosomes in the macromeres are clumped together at the surface of the egg, and are outside the plasma areas. There is a triaster in one of the micromeres, while the others appear normal.

Fig. 18. Same slide as preceding; 24-cell stage. All centrospheres instead of being at the surface, as in normal eggs, are at the deeper ends of the cells next to the spacious segmentation cavity.



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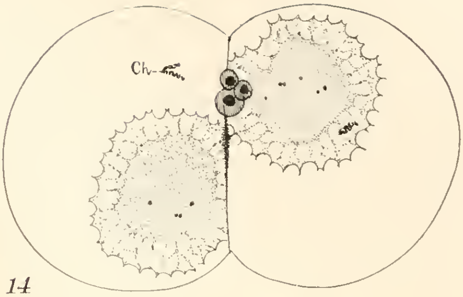

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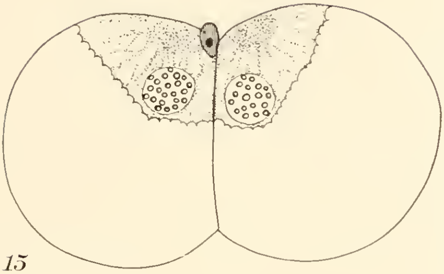

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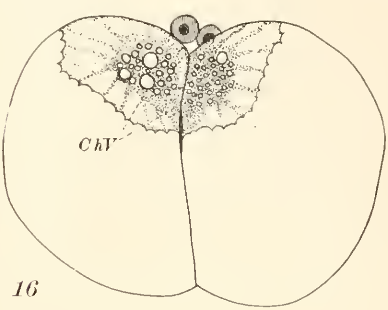

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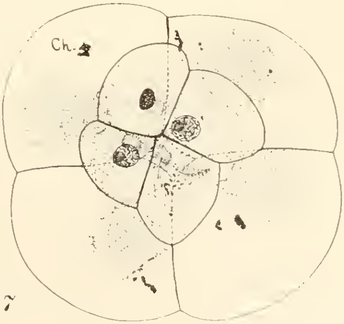

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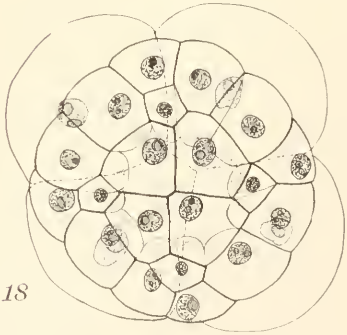

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EXPLANATION OF PLATE IV.

FIGS. 19–48 (with exception of Fig. 30) represent eggs which had been subjected to hypertonic solutions.

FIG. 19. No. 822. 2 per cent. NaCl, 16 hrs., normal sea-water 8 hrs. Numerous karyomeres were formed after maturation and before the first cleavage, presumably during the first cleavage mitosis.

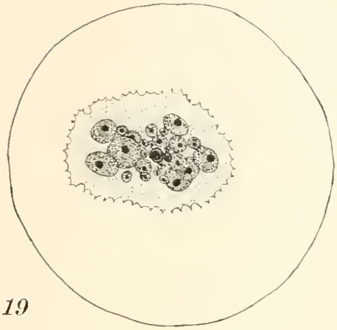
FIG. 20. Same slide as preceding. Karyomeres formed at the close of the first cleavage by chromosomes failing to unite. Centrospheres (*s*) unusually large.

FIG. 21. No. 837. $\frac{3}{4}$ per cent. KCl 9 hrs.; normal sea-water 35 hrs. Karyomeres irregular in size, shape and distribution.

FIG. 22. Same slide as preceding, with similar karyomeres.

FIG. 23. No. 822. 2 per cent. NaCl 16 hrs.; normal sea-water 8 hrs. Second cleavage abnormal, with karyomeres in two of the cells.

FIG. 24. No. 814. 2 per cent. NaCl 1 hr.; normal sea-water 17 hrs. Third and fourth cleavages abnormal, with karyomeres in most of the cells.



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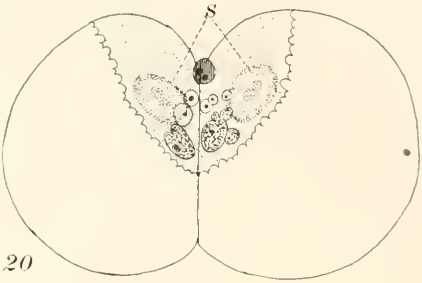

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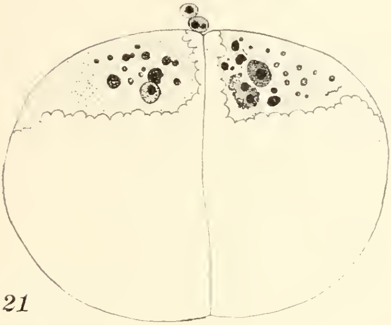

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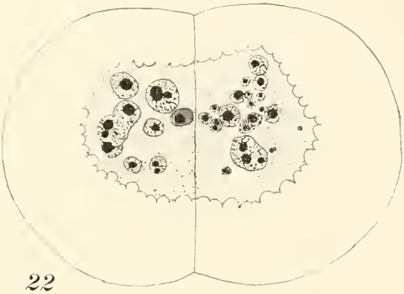

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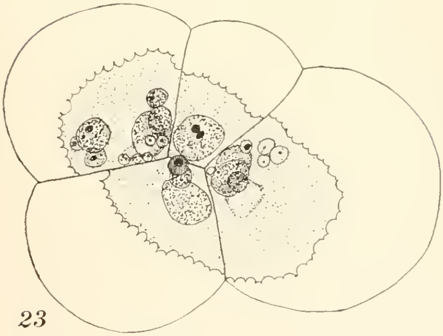

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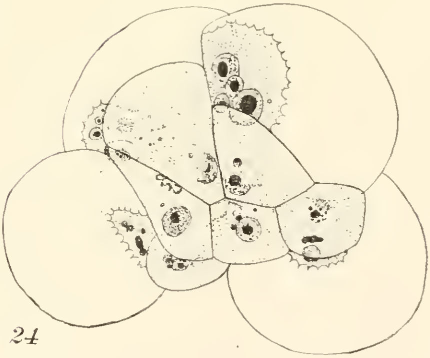

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Explanation of Plate V.

Fig. 25. No. 927. 2 per cent. NaCl 16 hrs., at close of third cleavage; normal sea-water 24 hrs. Numerous karyomeres in the cells.

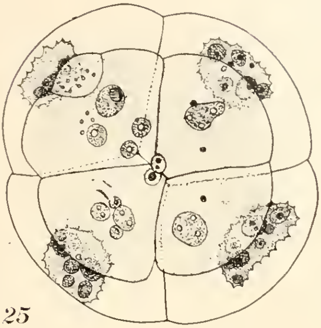
Fig. 26. No. 867. 8 per cent. $MgCl_2$ $\frac{3}{4}$ hrs., during third cleavage; normal sea-water $6\frac{1}{2}$ hrs. Numerous karyomeres in resting cells; in dividing cells, polyasters and scattered chromosomes.

Fig. 27. No. 972, same slide as Fig. 25. Karyomeres in all resting cells; polyasters and scattered chromosomes in dividing ones.

Fig. 28. No. 971. 2 per cent. NaCl 16 hrs., during third cleavage; normal sea-water 12 hrs. Similar to preceding.

Fig. 29. No. 828. 1 per cent. NaCl 2 hrs., during third cleavage; normal sea-water $6\frac{1}{2}$ hrs. Polyasters and chromatic connections between daughter nuclei.

Fig. 30. No. 956. Sea-water diluted with equal parts of fresh water 2 hrs.; normal sea-water 36 hrs. The nuclei in yolk-containing cells show many karyomeres.



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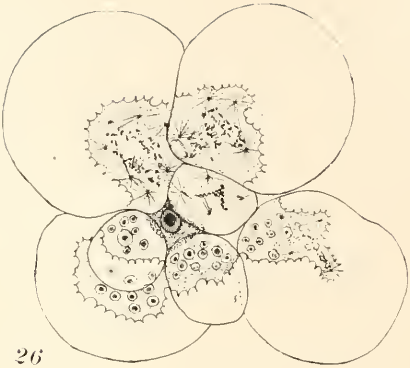

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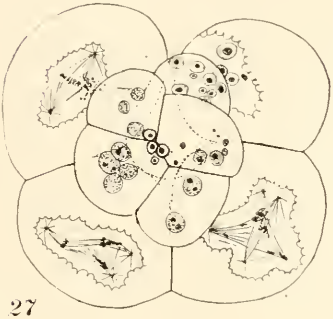

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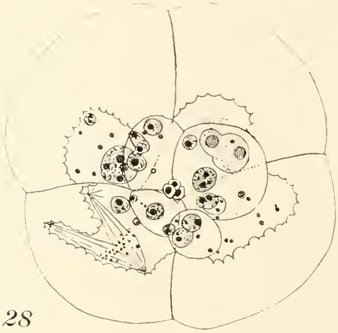

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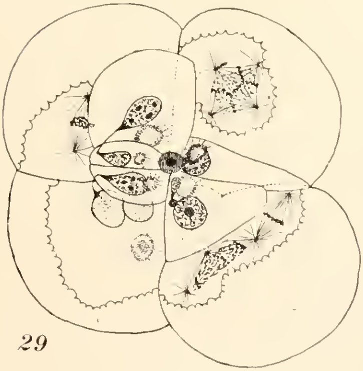

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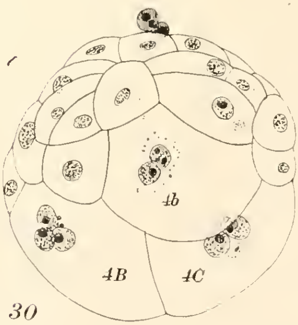

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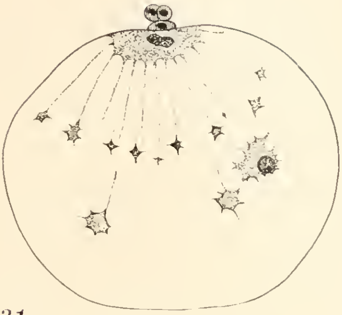
EXPLANATION OF PLATE VI.

FIG. 31. Normal egg showing cytasters sometimes present in eggs at the stage between the close of the maturation divisions and the beginning of the first cleavage. These cytasters are local aggregations of plasma along the lines of astral radiations.

FIGS. 32, 34, 35. Eggs from Exp. No. 805. 2 per cent. NaCl 4 hrs. Numerous cytasters are shown in different cell stages and division phases; in every case these cytasters are local aggregations of plasma along the lines of astral radiations, the remaining plasma being gathered around the nuclei or spindles.

FIGS. 33, 36. No. 998(3). 1 per cent. NaCl 5 hrs. The plasma is concentrated around the spindles, only small portions being left along the astral radiations in the 2-cell stage and none in the 4-cell stage. No cytasters are present after the 2-cell stage.



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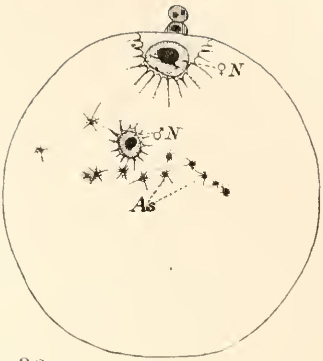

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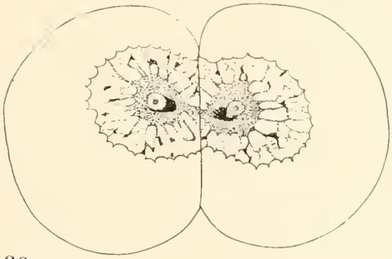

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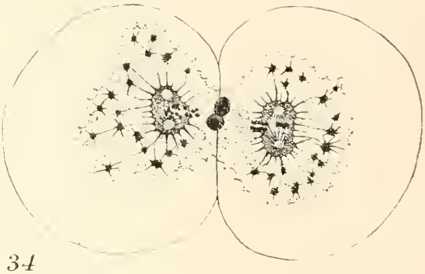

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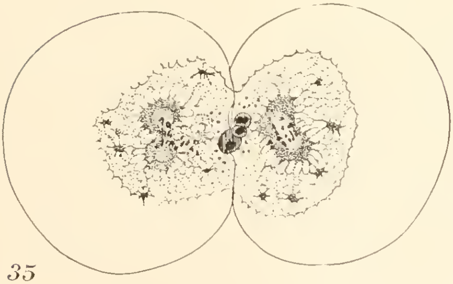

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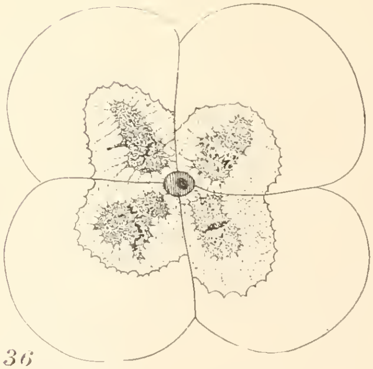

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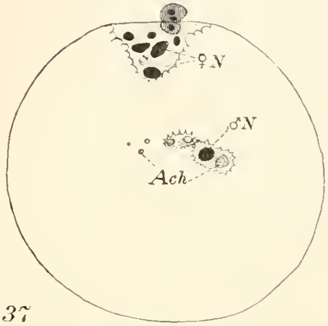
EDWIN G. CONKLIN.

EXPLANATION OF PLATE VII.

FIGS. 37–42. Various eggs from one experiment, viz., No. 809. 2 per cent. NaCl 15½ hrs. All figures show the chromatin in a densely chromatic mass and in all cases except Fig. 37, where the egg nucleus consists of several karyomeres, all the chromatin of each nucleus is aggregated into a single mass. On the other hand the achromatin consists of vesicles of different sizes which are more or less widely scattered, though a large achromatic vesicle is usually found adjoining a chromatic mass. This achromatin has not been squeezed out of the chromatic mass by the action of the salt solution, but the chromosomes of the daughter nuclei have been prevented from absorbing achromatin by the action of the salt solution.



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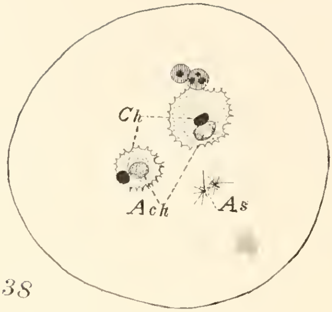

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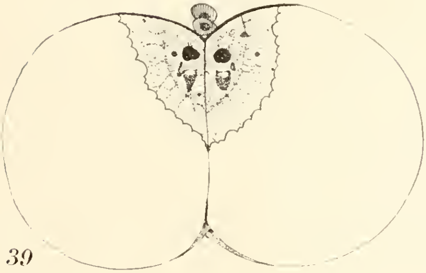

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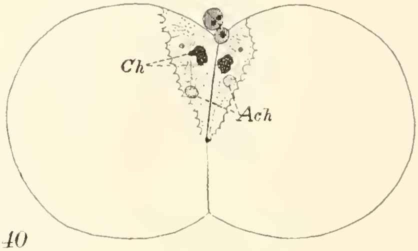

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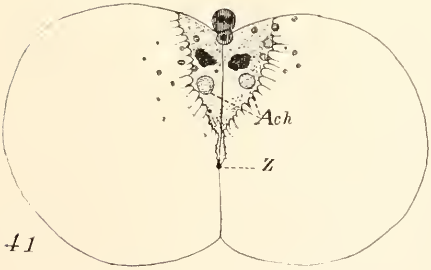

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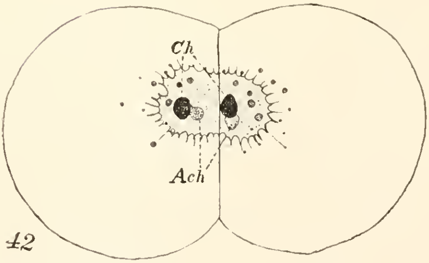
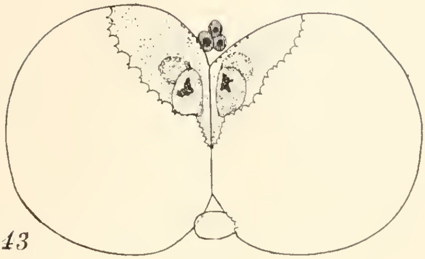


42

EDWIN G. CONKLIN.

Explanation of Plate VIII.

Figs. 43–48 are different eggs from the same experiment, viz., No. 810. 3 per cent. NaCl 15½ hrs. All the eggs were in the anaphase or telophase of the second cleavage at the time they were placed in the salt solution, and in all cases the chromosomes have remained in the form of a dense chromatic plate or mass which has not become vesicular. In Fig. 45, the spindle remnants between the chromosomal plates has become vesicular; in Figs. 46 and 47, the spindle area has become an elongated achromatic vesicle, within which lies the dense chromatic mass; in Figs. 43, 44, 48 the achromatic vesicle has become more nearly spherical in outline, while the chromatic mass is not quite so dense as in earlier stages. These figures show that when the chromosomes are prevented from absorbing achromatin and becoming vesicular nuclei, the achromatin of the spindle region may become a vesicle by the formation of a delicate achromatic membrane around itself.



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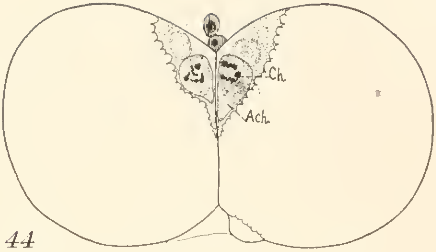

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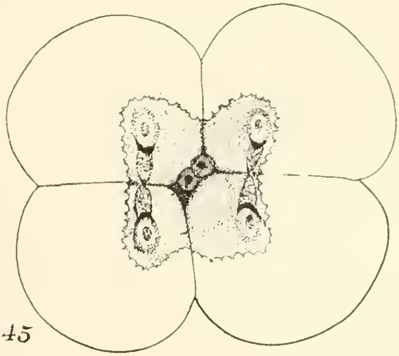

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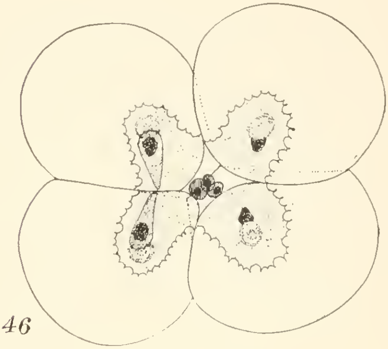

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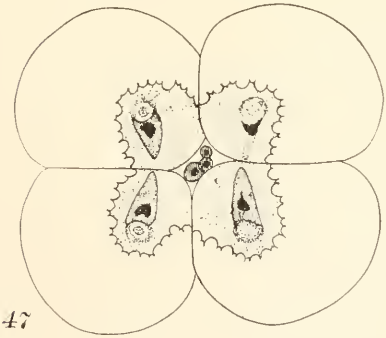

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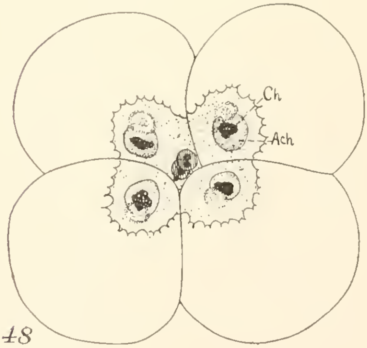

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EDWIN G. CONKLIN.

Explanation of Plate IX.

Figs. 49–60 represent eggs which had been subjected to diluted sea-water.

Figs. 49–54. No. 859. Sea-water 1 part, fresh water 2 parts, 1 hr.; normal sea-water 4 hrs. Eggs were treated with this diluted sea-water during the third and fourth cleavages, thus causing a scattering or stretching out of chromosomes along the spindle and the formation of chromatic connections between daughter nuclei. In cell D, Fig. 49, the division was stopped in the prophase, the nucleus being pear-shaped with the chromatin chiefly in the narrow upper end of the pear. It is significant that the long axis of the pear is in the direction of the spindle axis and that if the constriction were to separate the neck from the body of the pear, the daughter nuclei thus formed would be of approximately the same size as in normal eggs.

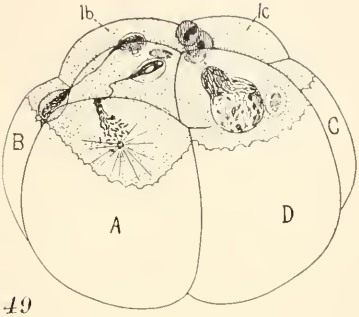
Fig. 51 shows more advanced stages of a similar process in which the chromatin is chiefly in the smaller, upper nuclei, the achromatin in the larger, lower ones.

Figs. 49 and 50 show certain nuclei in which the nuclear membrane remains intact though the chromosomes are arranged along a line or spindle connecting the two centrosomes.

Figs. 52–54. Eggs in which the third cleavage took place by a modified form of mitosis which left chromatic connections between daughter nuclei, and yet typical spindles for the fourth cleavage are present in some of the macromeres.

Fig. 52. In all four quadrants of this egg nuclear division at the third cleavage took place by modified mitosis, the daughter nuclei remaining connected by chromatic threads; in only two quadrants were micromeres formed, and the macromeres of these quadrants are now dividing by mitosis.

Figs. 53, 54. All daughter nuclei are connected by chromatic threads. Chromatin aggregates on the side of the nucleus next the centrosome (Fig. 54, *D*) and the chromatic connections between daughter nuclei run to the outer sides of the nuclei and spindles in the macromeres; the latter may be approximately normal, though the spindles may be out of proper position and the chromosomes more or less scattered.



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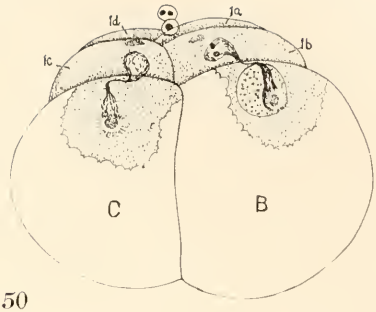
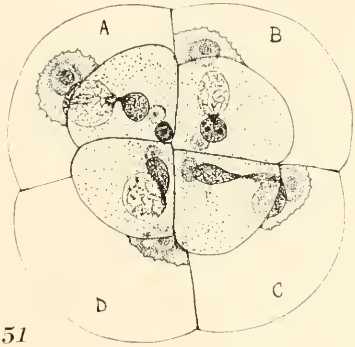

1d
1a
1c
1b
C
B

50



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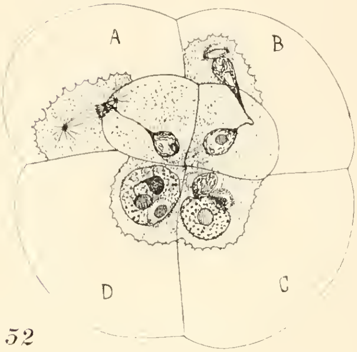

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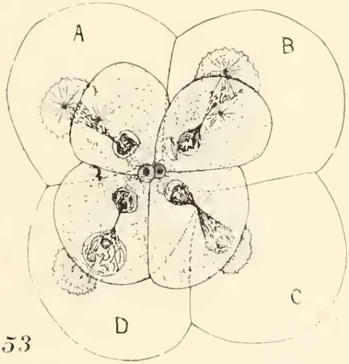

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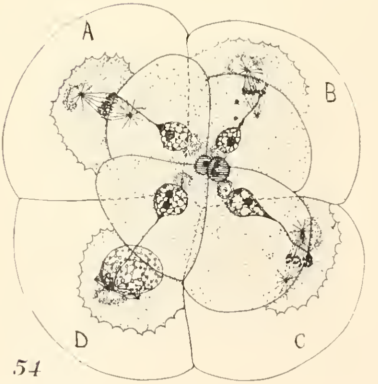

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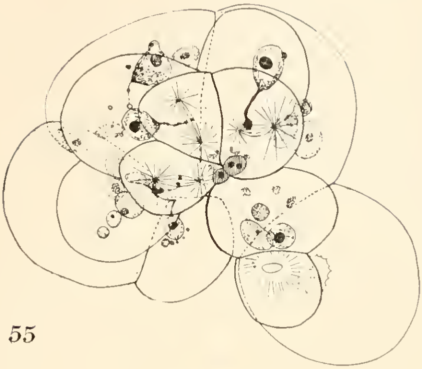
Explanation of Plate X.

Figs. 55–60. All from one experiment, No. 858. Eggs in 2–4-cell stage were left in sea-water diluted with equal parts of fresh water for two hours; then in normal sea-water fourteen hours. Remains of chromatic connections which were formed during the third cleavage are seen in Figs. 55, 56, 60. Other chromatic connections which were formed in later cleavages after the eggs had been returned to normal sea-water are shown in Figs. 57–60.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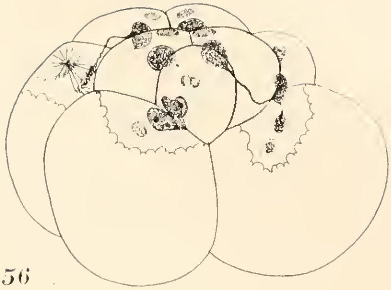
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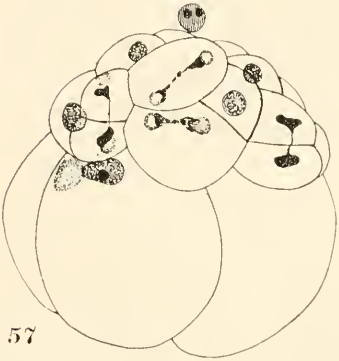
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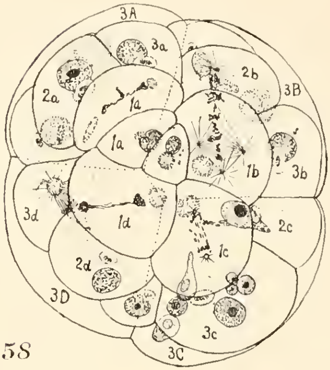
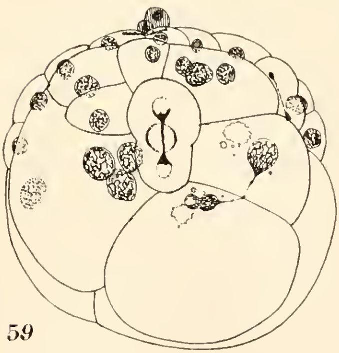

3A
3a
2b
3B
2a
1a
1a
1b
3b
3d
1d
2c
1c
2d
3D
3c
3C
58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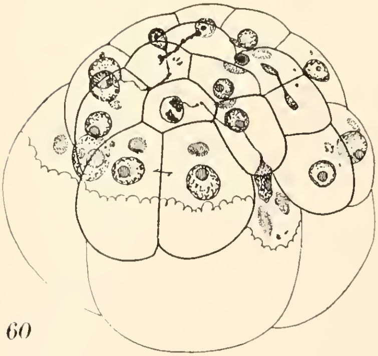
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THE INFLUENCE OF GENERAL ENVIRONMENTAL CONDITIONS ON THE PERIODICITY OF ENDOMIXIS IN PARAMECIUM AURELIA.

LORANDE LOSS WOODRUFF,

Osborn Zoölogical Laboratory, Yale University.

(Twelve figures.)

It is clear from the evidence submitted in previous papers that there are normal, minor, periodic fluctuations (rhythms) in the rate of reproduction of *Paramecium*,[1] and that at the low point in the division rate between two rhythms there normally occurs an intracellular reorganization process (endomixis).[2] The data previously presented also show that in *Paramecium aurelia* endomixis occurs, generally speaking, at intervals of about four weeks or about fifty generations, and that the synchronism of the process in different lines of the same race under identical conditions is remarkably exact.[3] But, as was stated incidentally, on the basis of our experience in working out the cytological phenomena of endomixis: "It is possible to retard or hasten the occurrence of the process by the character of the culture medium. For example, it may occur a few days earlier in animals not supplied daily with fresh culture fluid than in the regular lines." [4] Such being the case it is important to determine the influence of environmental conditions on the duration of the rhythms and the occurrence of endomixis.

The present paper comprises chiefly a study of the effects of what may be termed general changes in the culture conditions, such as markedly different culture media and temperatures on rhythms and endomixis. It seemed important to obtain a more definite background of knowledge of the influence of what perhaps may be called normal environmental changes before attempting to study the influence of, for example, specific chemical agents on the process.

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[1] Woodruff and Baitsell, "Rhythms in the Reproductive Activity of Infusoria," *Journ. Exper. Zoöl.*, XI., 4, 1911.

[2] Woodruff and Erdmann, A Normal Periodic Reorganization Process without Cell Fusion in *Paramecium*," *Journ. Exper. Zoöl.*, XVII., 1914; Erdmann and Woodruff, The Periodic Reorganization Process in *Paramecium caudatum*," *Journn. Exper. Zoöl.*, XX., 1916; Woodruff, "Rhythms and Endomixis in Various Races of *Paramecium aurelia*," Biol. Bull., XXXIII., 1917.

[3] Cf. Woodruff and Erdmann, 1914, Tables 1, 2, 3.

[4] Woodruff and Erdmann, 1914, p. 485.

It is assumed in the present paper that the reader is familiar with the earlier work on *Paramecium* which has been published from the Yale Laboratory.

Material and Methods.

The organisms employed in the work were from pedigreed cultures of *Paramecium aurelia*. Some of these had been under culture conditions for long periods—one for more than 5,000 generations—while others were started with this work in mind. Each of the five races used was started originally with a 'wild' individual which was secured from a locality far removed from that of the others, so that representative diverse material was afforded. The early life history of each of these cultures has been presented in connection with other work and the reader is referred to these papers for further details.[1]

All the organisms studied have been carried in pedigreed subcultures isolated from the respective main cultures of the various races, and since the method of conducting such cultures has been described many times in earlier papers it need not be repeated in detail here. Suffice it to say that the method involves the isolation of one or more animals from each line of every subculture practically every day and in addition, for the work in hand, the preservation and cytological study of some of the stock animals left over at the time of the daily isolations. In this way the occurrence of endomixis has been determined.

The main cultures have been carried on the 'varied' culture medium which we have found for ten years so favorable in breeding *Paramecium*.[2] This consists of infusions of vegetable and animal debris collected from time to time from laboratory aquaria, ponds, etc., and, of course, thoroughly boiled and allowed to attain room temperature before being used. Some of the subcultures directly involved in the experiments have been bred on this medium. Others have been bred on the beef-extract medium which we have employed in other work on *Paramecium*,[1] or on other media which will be described in connection with the individual experiments.

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[1] Cf. especially Woodruff, Biol. Bull., XXXIII., 1917.

[2] Woodruff: "The Life Cycle of *Paramecium* when Subjected to a Varied Environment," *American Naturalist*, XLII., 1908.

The subcultures which have been the basis of the present work may be tabulated as follows:

SUBCULTURES.

A (from Main Culture I) Oct. 15, 1914, to Feb. 12, 1916. (485 days.)
4675th to 5592d generations.
AE (from IE)[2] Oct. 15, 1914 to Aug. 12, 1915. (Twice restarted during the 300 days.)
4637th to 5079th generations.
O (from III) Oct. 15, 1914 to Nov. 20, 1915. (Once restarted during the 400 days.)
17th to 775th generations.
B (from IV) Jan. 8, 1915 to Jan. 14, 1916. (372 days.)
3d to 609th generations.
M (from V) July 17, 1915 to Feb. 23, 1916. (222 days.)
3d to 550th generations.
W (from VI) Aug. 12, 1915 to Jan. 14, 1916. (152 days.)
3d to 302d generations.

The data are presented chiefly by graphs of the division rate of the various subcultures. These are plotted by averaging the daily rate of division of the several lines of the respective subcultures and then again averaging this for five-day periods. The figures 1, 2, 3 represent divisions and 10, 20, etc., indicate the number of the five-day periods. An *E* shows that endomixis was observed during the five-day period. Inclusion of a part of the curve within brackets indicates that the cells were not studied cytologically during this time. Cf. Figs. 1 and 7. Since so much depends on these graphs and the five-day periods which they comprise it may be well to repeat a statement made in a previous paper:[3]

---

[1] Woodruff and Baitsell, "The Reproduction of *Paramecium aurelia* in a 'Constant' Culture Medium of Beef Extract," *Journ. Exper. Zoöl.*, XI., 1, 1911.

[2] *IE* is a subculture isolated from Main Culture I. in October, 1913.

[3] Woodruff and Erdmann, *Journ. Exper. Zoöl.*, 1914, p. 477.

"The five-day period was adopted in the presentation of our results because this was the method of constructing the

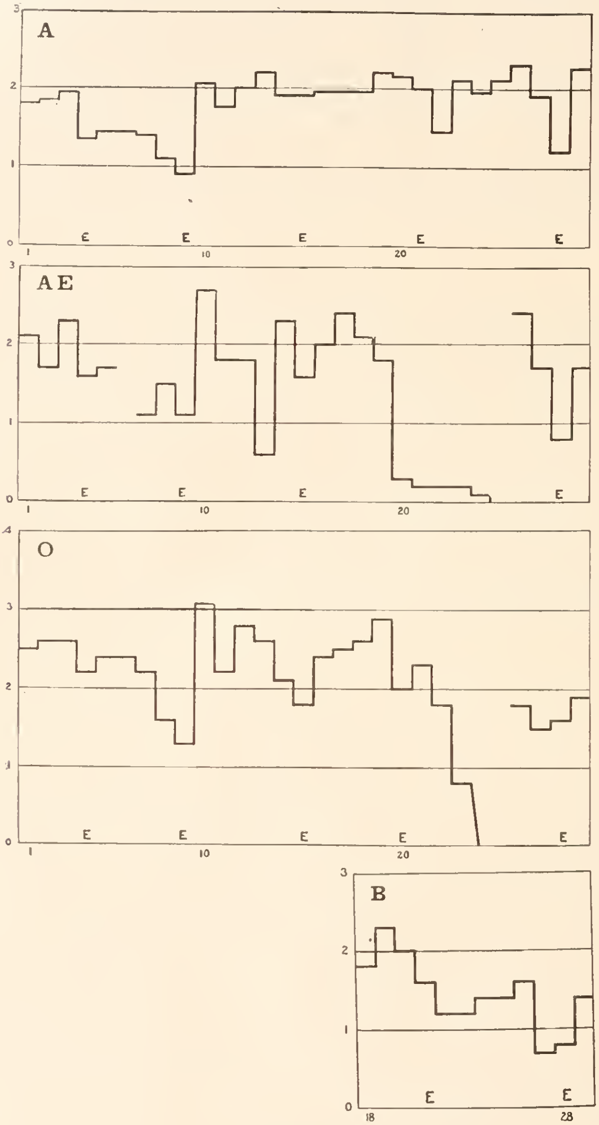

FIG. 1.

graph emphasized in the original study of rhythms in this culture.[1] It is realized, of course, that a five-day period is largely an arbitrary one and that the ideal graph would present the momentary changes in the metabolism of the cell. Data for such a curve being absolutely impossible to secure, it might seem at first glance that the daily record of division would approach most nearly to this ideal condition. As a matter of fact, the twenty-four-hour period is as arbitrary as the five-day period when it is considered that this is a long period when compared with the metabolic changes in the cell and that the daily record, made at approximately 11 A.M., would merely give the divisions actually completed during the previous twenty-four hours. For example, let us assume that, at the time of isolation, two animals are present, representing one division during the previous twenty-four hours. The record for that day is one division. One animal is then isolated and it divides within an hour and each of the resulting cells again divide twice before the next isolation. The record for this second day is three divisions, thus the record for the two days shows a different division rate for each day, *i. e.*, one division against three divisions, whereas a more true, but not a perfect, picture of the state of affairs is given by the statement that four divisions occurred in forty-eight hours. One might follow this argument to its logical conclusion and assume that the best method of presentation would be to average for considerable periods, *e. g.*, 10 or 30 days, but this obviously would tend to obliterate any fluctuations in the rate which are not of relatively long duration. The adoption of the five-day period was made in recognition of both of these contingencies, and it was of a duration particularly well suited to show the effect of the process on the reproductive rate, because the process extends over about nine cell divisions or a period of about six days. Consequently the effect of the process makes itself evident in the five-day plot. Certain apparent irregularities in the coincidence of the phenomena are, from an actual study of all the data at hand, clearly due to the fact that the five-day period is not ideal."

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[1] Woodruff and Baitsell, *Journ. Exper. Zoöl.*, XI., 1, 1911.

Experiments—Series I.

The experiments of Series I. may be outlined as follows:

A. Study of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred under the same *varied* culture conditions.

B. Study of the periodicity of rhythms and endomixis in

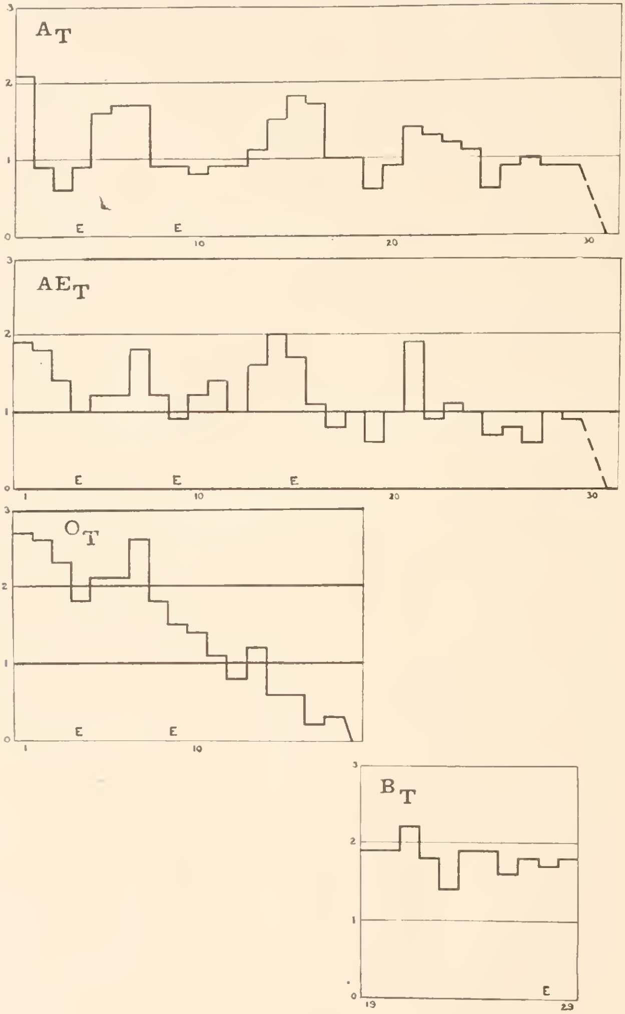

FIG. 2.

different races of *Paramecium aurelia* when bred under the same *constant* culture conditions.

C. Study of the periodicity of rhythms and endomixis in the *same* races of *Paramecium aurelia* when bred under the *varied* and under the *constant* culture conditions.

These experiments were carried on from October 15, 1914, to March 10, 1915; a period of 145 days. The subcultures employed were *A*, *AE*, and *O* throughout the work and *B* from its isolation on January 8, 1915, to the end. Each of these subcultures represents a different race of *Paramecium aurelia*, except *A* and *AE*, both of which were originally derived from the same stock, Main Culture I., about 1,000 generations before.

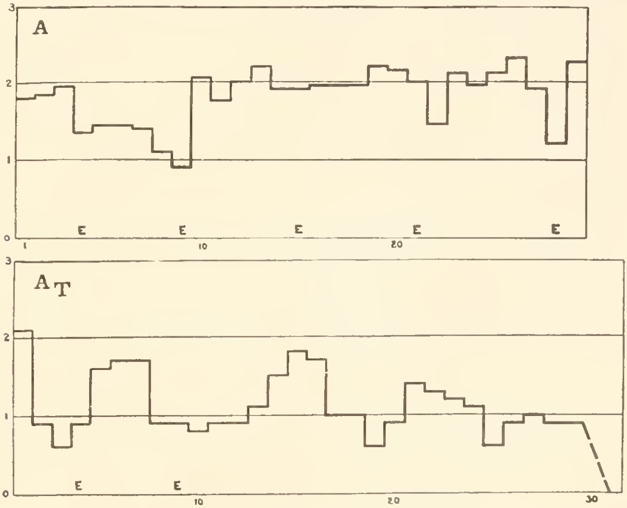

FIG. 3.

All of these subcultures were subjected to a varied culture medium and to the ordinary fluctuation in room temperature.

Sister subcultures (designated *At*, *AEt*, *Ot* and *Bt*), were isolated line by line from the above cultures at the start of the experiment and were subjected to a constant culture medium of beef extract. The temperature was maintained relatively constant at about 26° C. in a thermostat.

A.

Study of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred under the same *varied* culture conditions.

Fig. 1 presents the graphs of the division rate of subcultures *A*, *AE*, *O* and *B* throughout this experiment. A study of the figure shows that *A* underwent endomixis at periods Nos. 4, 9, 15, 21 and 28. *AE* showed endomixis at period 4 and then was lost by an accident. A new *AE* was started by isolating line by line from *AEt* (which had been branched from it 25 days before and subjected to the constant culture conditions). *AE* then showed endomixis at the periods 9 and 15 and died out in period 25 without again undergoing endomixis. Still another *AE* was isolated immediately from *AEt* and had endomixis during the 28th period. A similar survey of the graph of *O* shows that endomixis took place at periods 4, 9, 15 and 20. Subculture *O* died at period 25 from causes unknown. It was restarted from the main culture of this race at once and had endomixis at period 28. Subculture *B* was begun in period 18, that is immediately on the isolation of the race from 'wild' material. Endomixis occurred at periods 21 and 28.

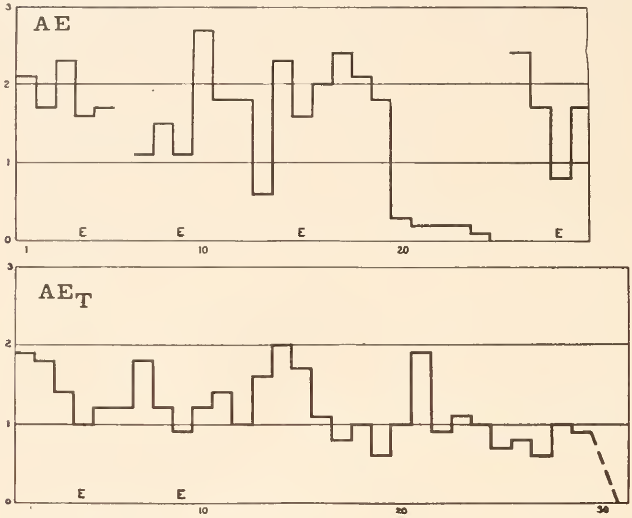

FIG. 4.

Tabulation of these data shows that endomixis occurred in these subcultures as follows:

A at periods	4	9	15	21	28
AE " "	4 lost	9	15	0 died	28
O " "	4	9	15	20 died	28
B " "	–	–	—	21	28

This experiment, in which different races were bred under the same varied culture conditions, shows an almost perfect synchronism of endomixis in all the races; that is, in *A* bred throughout without interruption on the varied medium; in *O* bred until its death near the end of the experiment on the same medium and again in the new *O* isolated from the main culture; in *B*, which was isolated from 'wild' material during the progress of the work; and finally in *AE*, which was restarted twice from a sister culture bred under "constant" environmental conditions.

The most reasonable conclusion, on the basis of this experiment, to account for the fact that the different races immediately showed endomixis synchronously, is that the general culture conditions initially influenced the appearance of the process; that is, brought about its consummation a few days earlier or later than it would have appeared under the former environment of the races, and that, once established, the rhythmic period characteristic of the species persisted and maintained the synchronism of endomixis. If this conclusion is justified then it must be assumed that the synchronism of *B* is due to the chance isolation of this race at just the same period in the rhythm which *A*, *AE* and *O* were at at the time, or so near this period that the environmental change immediately made it coincide with that of the other races. The alternate hypothesis would be that there is in all races of this species a definitely established synchronism which holds under all normal environmental conditions. But such a theory would require more than one series of experiments to render its discussion profitable!

B.

In this set of experiments a study was made of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred under the same practically constant culture conditions, as already described. The results are shown graphically in Fig. 2.

Subcultures *At* and *AEt* (both derived from the same race, I, about 1,000 generations previously) showed endomixis in preserved specimens at periods 4 and 9 and *AEt* also at period 15. Neither culture again underwent the process during a period of over 100 days before it died, though the characteristic 'rhythms' in the division rate are apparent during this time. It is possible that the process did occur at about periods 19 and 25 (cf. Fig. 2) and was overlooked, but I believe that this is highly improbable in view of the thoroughness of the search.

Subculture *Ot* underwent endomixis at periods 4 and 9 and then died without repeating endomixis at the next expected period (cf. Fig. 2).

Subculture *Bt*, as the graph shows, had endomixis at the 28th period, just before the experiment was concluded. From the character of the curve it would be expected at period 23, but it was not observed.

The endomictic periods of the four subcultures (3 races) of *Paramecium* in this experiment may be tabulated as follows:

At at periods.........	4	9	0	0 died
AEt " "	4	9	15	0 "
Ot " "	4	9	0	– "
Bt " "	–	–	–	28

These experiments show three points of considerable interest. In the first place they corroborate, for races bred under constant culture conditions, what was found in the same races when bred under varied culture conditions in the experiment already described. That is, *At*, *AEt* and *Ot* show a perfect synchronism of endomixis, and this is most reasonably explained by assuming that the general culture conditions, at least initially, influenced the appearance of endomixis and that, once established, the rhythmic period characteristic of the organism persisted. *Bt* affords no data for comparison with the other races of this set of cultures, as they had ceased to show endomixis before *Bt* was started.

A second point of importance is that the 'rhythms' in the division rate are to a certain extent independent of endomixis—that is of the definitive series of nuclear phenomena—because the rhythms persist for a while in the absence of the morphological changes. One may suggest that the rhythms in the culture are an expression of the physiological conditions antecedent to the definitive onset of the nuclear changes—in other words that the cell has undergone the preliminary stages of endomixis which ordinarily call forth the observable nuclear changes but that in the cases in hand the latter were never realized. This is equivalent to making the term endomixis coextensive with the term rhythm—the term rhythm denoting the physiological effect as indicated in the reproductive activity, while the term endomixis covers all the underlying physiological changes together with the definitive nuclear phenomena characteristic of the low point in the division rate. The term endomixis was *not* used in that broad sense when first employed by Woodruff and Erdmann and it is unprofitable to extend it now. Applying the term strictly to the complicated reorganization process of the cell does not deny the undoubted fact that these changes are but the expression of the climax of a series of physiological phenomena which probably extend back to the consummation of the previous endomictic period. So, it seems to be a more or less academic question whether rhythms and endomixis are independent. Certainly the rhythms occur for a while without endomixis in *sensu stricto*—but in all such cases the culture has died before very long.

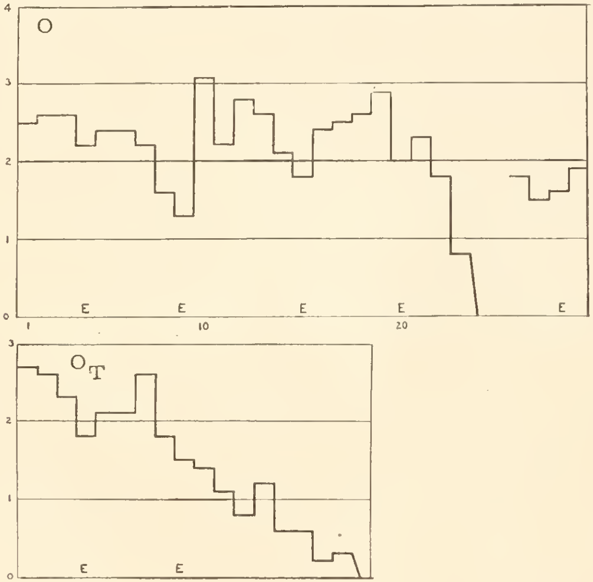

FIG. 5.

This leads to the third point of interest which is the death of the cultures after the cessation of endomictic phenomena. This is true in each instance: *At*, *AEt*, and *Ot* (cf. Fig. 2). The data from these experiments perhaps are not sufficiently extensive to make sure that this is not a case of *post hoc sed non propter hoc*, but they make it highly probable that endomixis is necessary for the continued life of the race.

C.

The data which have been presented in the study of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred under varied and under constant culture conditions, may now be analyzed from the point of view of the periodicity of these phenomena in the *same* races under varied and under constant culture conditions, since experiments *A* and *B* of this series were conducted simultaneously.

This analysis is readily made by a study of Figs. 3, 4, 5 and 6, which consist of a combination of the graphs already presented from the other point of view. The graphs are paired, one above the other, so that identical five-day periods coincide. For example, in Fig. 3, period 10 of *A* is directly above the same period of *At*. Since the endomictic periods of all these cultures have just been considered, it is only necessary to tabulate them for reference:

A underwent endomixis at periods....	4	9	15	21	28
At " " " "	4	9	0	0	0 died
AE " " " "	4	9	15	0 died	28
AEt " " " "	4	9	15	0	0 died
O " " " "	4	9	15	20 died	28
Ot " " " "	4	9	0	—	died
B " " " "				21	28
Bt " " " "				0	28

From this table it is apparent that the synchronism of endomixis is practically perfect in the *same* races when bred under *different* environmental conditions. The first two experiments showed that it was the same for *different* races when bred under the *same* environmental conditions whether varied or constant. Therefore this experiment corroborates and broadens the conclusions derived from the former ones and shows clearly that the periodicity of endomixis is largely independent of the character of the culture medium—the general environmental conditions—within the rather wide limits in which it has been varied in parts *A*, *B* and *C* of this series of experiments. At most the culture conditions initially influence the appearance of endomixis. In other words, the organism is set, so to speak, to undergo endomixis approximately once a month and this it does under any more or less favorable environmental conditions. A sudden change, however, of these conditions may bring about endomixis slightly earlier than it otherwise would have occurred but after this the usual rhythmic period of the species is maintained.

Experiments—Series II.

The experiments of this series may be outlined as follows:

A. Study of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred in a relatively *large* amount of culture medium supplied fresh *daily*.

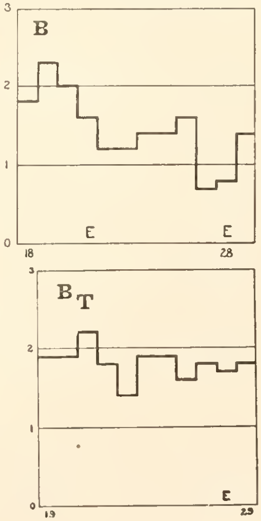

FIG. 6.

B. Study of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred in a relatively *small* amount of culture medium changed on *alternate days*.

C. Study of the periodicity of rhythms and endomixis in the *same* races of *Paramecium aurelia* when bred in a relatively *large* amount of culture medium supplied fresh *daily*, and in a relatively *small* amount of culture medium changed on *alternate days*.

These experiments were begun on July 2, 1915, and continued until February 22, 1916. Five different races of the organism were employed. Three of them (*A*, *O*, and *B*) were the same subcultures which were used in the experiments of Series I., and which had been continued during the interim—that is, from March, 1915, to July, 1915. Therefore the numbering of the five-day periods was continued from the earlier work and the first period of the present series is number 53. Two new races were secured from diverse localities as already described and subcultures from these were begun and made a part of this experiment at once. These subcultures were designated *M* and *W*.

All these subcultures were supplied daily with the regular varied culture medium. Sister subcultures (designated *As*, *Os*, *Bs* and *Ms*) were isolated line by line from the above-mentioned subcultures at the start of the work and were subjected to the same varied culture medium but the amount was reduced to one half that supplied to the parent subcultures and the medium was changed at intervals of 48 hours instead of 24 hours.

It has been shown in previous papers,[1] that the rate of reproduction of *Paramecium* is markedly influenced by the volume and the freshness of the culture medium. This was found to result from the accumulation of the excretion products, in view of the fact that a medium which contains the excretion products of a heavy growth of paramecia has a decidedly depressing effect on the division rate of this organism.

Accordingly it seemed that the excretion products of *Paramecium* afforded the most natural means of quickly modifying the division rate in order to determine the effect of this on the rhythms and endomictic periods. Obviously the experimental conditions involve two variables—excretion products, and their effect, the lowering of the fission rate—so that the specific influence of one or the other cannot be determined from the data given below. But that is not of importance in the present work which is merely an endeavor, as already stated, to determine the effect of normal environmental changes.

A.

This set of cultures was carried on in order to study the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred in a relatively *large* amount of varied culture medium supplied fresh *daily*. The experi-

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[1] Woodruff, "The Effect of Excretion Products of *Paramecium* on its Rate of Reproduction," *Journ. Exper. Zoöl.*, X., 1911; Woodruff, "The Effect of Excretion Products of Infusoria on the Same and on Different Species, with Special Reference to the Protozoan Sequence in Infusions," *Journ. Exper. Zoöl.*, XIV., 1913.

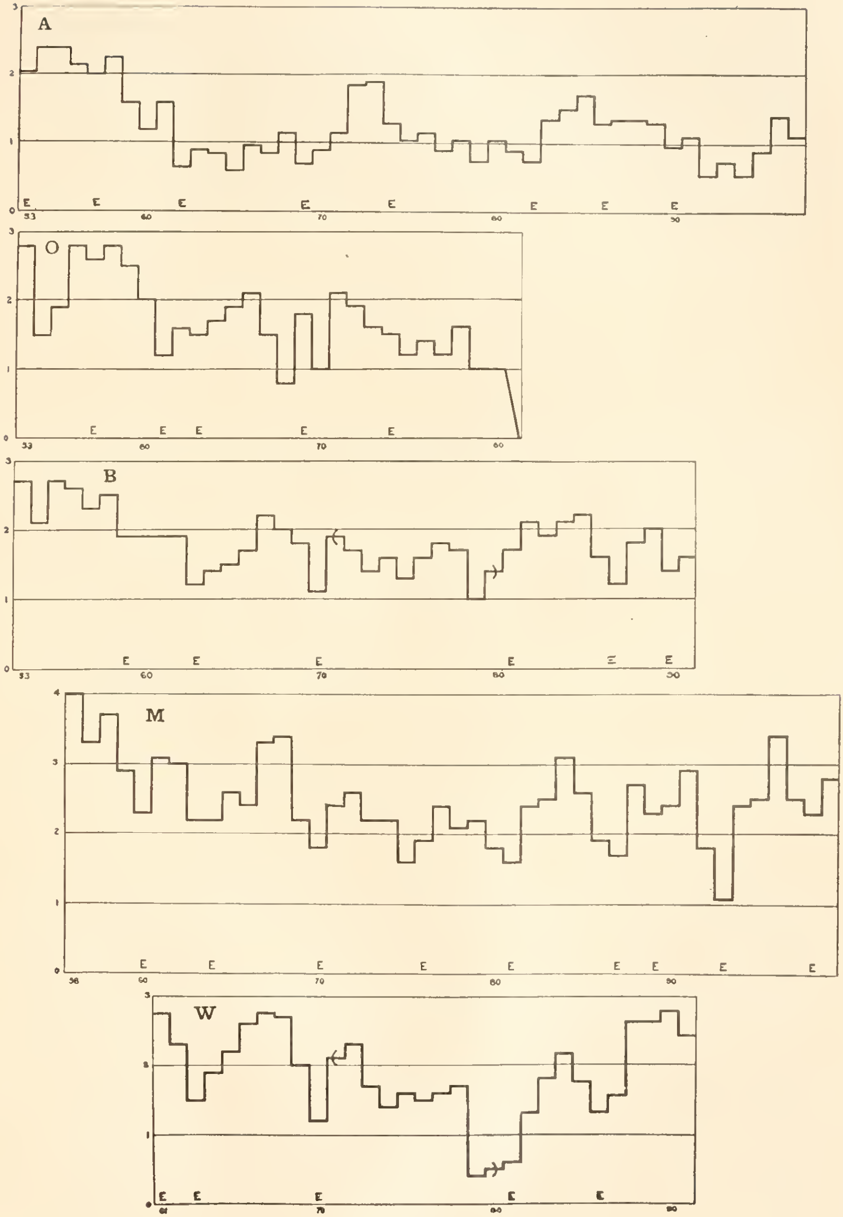
A
3
2
1
0
E E E E E E E E
53 60 70 80 90
O
3
2
1
0
E E E E E
53 60 70 80
B
3
2
1
0
E E E E E E
53 60 70 80 90
M
4
3
2
1
0
E E E E E E E E E
58 60 70 80 90
W
3
2
1
0
E E E E E
61 70 80 90

FIG. 7.

ment is essentially a repetition of experiment *A* of Series I (cf. p. 443) since three races (*A*, *O* and *B*) and the character and amount of culture medium are the same as employed before. The results, therefore, now will be analyzed from the viewpoint of Series I. and later from that of the present series.

The results are shown graphically in Fig. 7 from which it is evident that:

A underwent endomixis at periods . . .	53	57	62	69	74	82	86	90		
O “ “ “ “ . . .		57	61–63	69	74					
B “ “ “ “ . . .		59	63	70	[1]	81	87	90		
M “ “ “ “ . . .		60	64	70	76	81	87	89	93	98
W “ “ “ “ . . .			61–63	70	[1]	81	86			

A study of this table and the graphs which it summarizes shows again practically the same periodicity of endomixis in diverse races on the varied culture medium as was observed in experiment *A* of Series I. The synchronism is not quite as exact as in the former experiment but, considering all the unknown and uncontrollable variables in such a long experiment, it clearly offers further support for the conclusion that rhythms and endomixis are essentially independent of environmental conditions, and that the culture conditions merely influence initially, if at all, the appearance of endomixis, and that once established the rhythmic period characteristic of the species is maintained within rather narrow limits.

B.

This set of experiments involves a study of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred in a relatively *small* amount of culture medium changed on *alternate days*. The cultures used, as already stated, were *As*, *Os*, *Bs* and *Ms* and their behavior with respect to the process under consideration is given in Fig. 8. A study of this graph shows that endomixis was observed in:

As at periods.	59	61	65	70	[1]	0	85	90
Os “ “	59	0	64	69	[1]	82	86	
Bs “ “	59	0	64	70	died			
Ms “ “	59	62	64	70	[1]	82	87	91

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[1] The animals were not studied cytologically during this period.

Again the synchronism is very marked throughout the work, while during the initial period (59) it is perfect. The explanation of the occurrence of endomixis in every culture during the 59th period is undoubtedly due to the *marked* change of culture conditions to which these subcultures were subjected, when the experiment was initiated in period 58, by isolation of the 's' subcultures from their respective main cultures on the varied culture medium changed daily (cf. Figs. 9 to 12). This will be considered in connection with the following experiment, but obviously it is in accord with the results obtained by Woodruff and Erdmann[1] who noted in studying various *lines* of one race that the reorganization process "may occur a few days earlier in

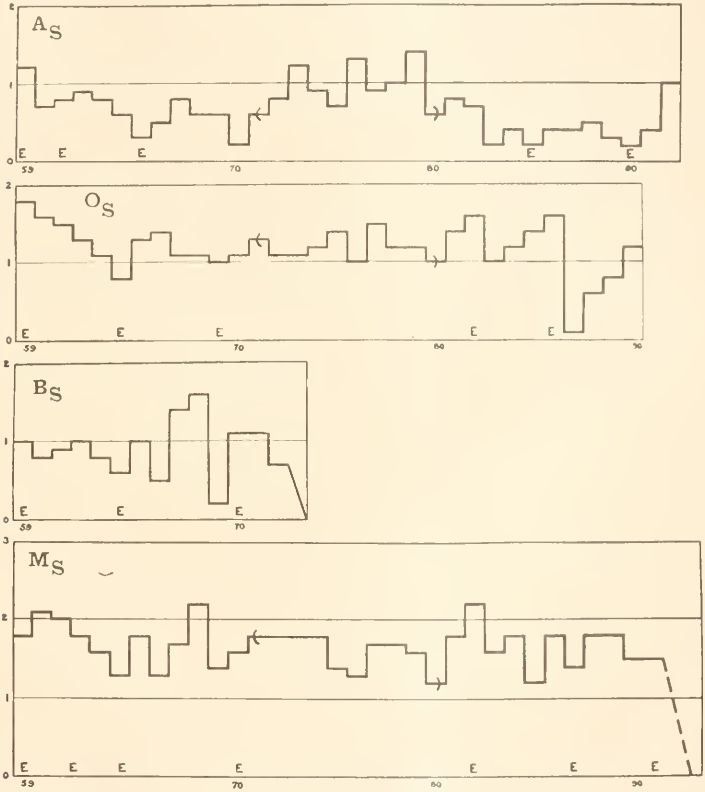

FIG. 8.

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[1] *Journal of Experimental Zoölogy*, 1914.

animals not supplied daily with fresh culture fluid than in the regular lines."

It is apparent then from this experiment that the same general conclusion which was derived from all the previous ones is again justified, viz., the culture conditions may, at most, initially influence the appearance of endomixis; but once established the rhythmic period characteristic of the species is maintained with great exactness, resulting in a remarkable synchronism of the process in the different races. General normal environmental changes obviously do not permanently alter the fundamental inherent rhythmic periods of the organism.

C.

A study now can be made of the phenomena under consideration in the *same* races of *Paramecium aurelia* when bred in a relatively large amount of culture medium supplied fresh daily, and in a relatively small amount of culture medium changed on alternate days. This involves, obviously, the comparison of the results from the two previous experiments since these were conducted simultaneously and afford the requisite data. Therefore the culture graphs of these two experiments are presented, one above the other, so that identical five-day periods coincide, in Figs. 9, 10, 11 and 12.

It is to be noted that *As* and *Os* were branched from *A* and *O* very soon after endomixis had occurred in the latter cultures; *Bs* came from *B* during the actual occurrence of endomixis; while *Ms* was branched from *M* toward the end of a rhythm as the subsequent appearance of the process in *M* at period 60 shows.

The four figures mentioned and the following tabulation of the periods in which endomixis occurred in the various subcultures shows the synchronism of the reorganization process in all the pairs of cultures under the markedly different environmental conditions. It is difficult to say whether this coincidence of the process is more exact between different races under the same culture conditions or between the same races under different culture conditions, because most of the variations are so small that they fall well within the limits of error involved in the five-day plotting method, etc. (cf. p. 440).

A at periods..	53	57	62	?	69	74	82	86	90
As " " ..		59	61	65	70	[1]	0	85	90
O " " ..		57	61	63	69	74	died		
Os " " ..		59	0	64	69	[1]	82	86	
B " " ..		59	0	63	70	[1]	81	87	90
Bs " " ..		59	0	64	70	died			
M " " ..			60	64	70	76	81	87	89
Ms " " ..		59	62	64	70	[1]	82	87	91

Thus clearly, in the long run, the *s* environment had no effect on the periodicity of the process. But, as pointed out before, endomixis appeared without exception in the four *s* cultures immediately upon their isolation from the respective parent cultures and on subjection to the stale culture fluid. Therefore, now that it is possible to compare parent and daughter lines, the obvious conclusion from the data is that endomixis was brought about earlier (except in *Bs* which was started during endomixis) by the changed cultural conditions; that is, earlier than it appeared in the parent cultures. This result, it may be noted, substantiates the conclusion from the former experiments that the remarkably exact synchronism of endomixis of various races under the most diverse environmental conditions is due to a slight initial influence on the occurrence of the process.

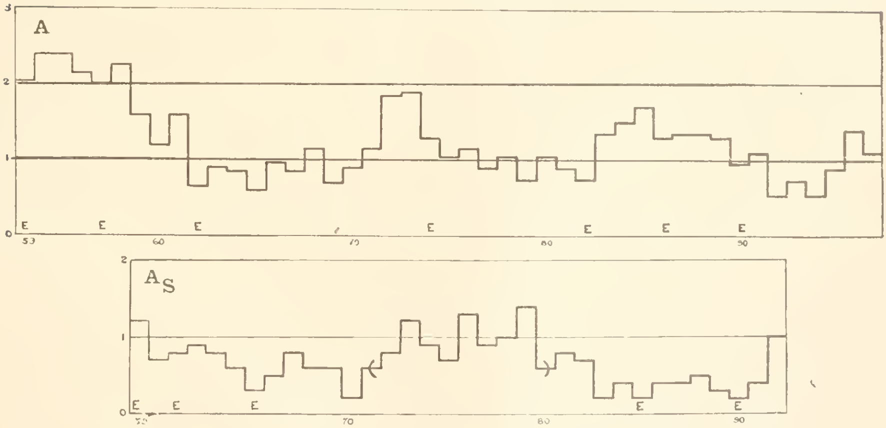

FIG. 9.

Figs. 9, 10, 11 and 12 show that the treatment to which the *s* cultures were subjected resulted in a distinctly lower rate of division—on the average about three quarters of division per day lower than in the cultures subjected to the fresh culture medium, etc. This is without doubt due, as already discussed, in large part at least to the accumulated excretion products in the *s* series. But whatever the cause, the experiment affords an opportunity to study the effect of naturally changed conditions, involving a lowered fission rate, on the periodicity of rhythms and endomixis.

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[1] Not studied cytologically during this period.

Now since the *s* subcultures divided at a much lower rate than the parent cultures, and since endomixis appeared fairly synchronously in parent and *s* sets, it is obvious that endomixis consistently appeared in the *s* subcultures within a smaller number of generations. In other words, the treatment of the *s* series apparently resulted merely in reducing the number of cell divisions in a given time and had practically no effect (except in the first period) on the occurrence of endomixis. On the basis of this set of cultures, then, endomixis is to a certain extent independent of the number of generations and more closely related to a time factor, if such an expression may be employed.

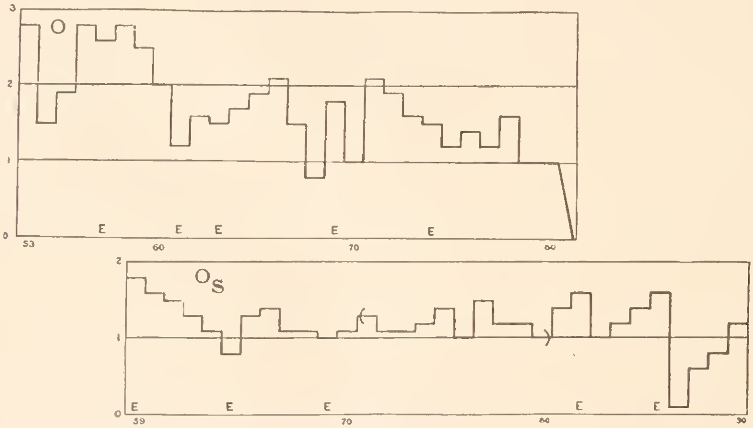

FIG. 10.

The *B* pair of cultures affords a fairly typical example (Fig. 11). In *B* endomixis occurred at periods 59, 63 and 70; while in *Bs* it occurred at periods 59, 64 and 70. Thus the length of time in days between successive reorganizations is 20 and 35 in *B*, and 25 and 30 in *Bs*. Therefore in both cultures the same number of days (55) elapsed from one endomixis to its second following occurrence. On the other hand in *B* the process was in progress at the 335th, 370th and 435th generations, that is at intervals of 35 and 65 generations, while in *Bs* it took place at the 335th (when *Bs* was isolated from *B*), 360th and 380th generations, or at intervals of 25 and 20 generations. Therefore in *B* 100 generations were attained from one endomictic period to the second following occurrence of the process; while in *Bs* only 45 generations occurred during the same period. To repeat: the experimental treatment apparently simply reduced the number of generations during the 100 days.

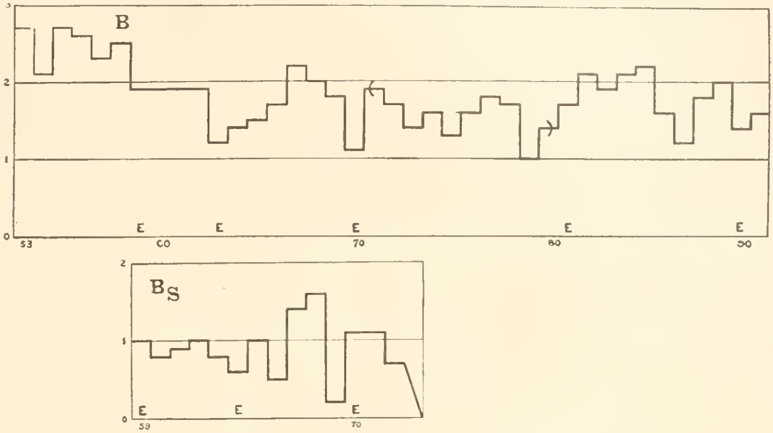

FIG. 11.

This same general result was obtained in Set *C* of Series I., though of course here the lowering of the division rate was the result of other causes. Figs. 3 and 4 show that endomixis occurred in the fourth period of the four subcultures, *A* and *At* and *AE* and *AEt*, although the rate of division and therefore the number of generations was less in *At* than in *A*, and less in *AEt* than in *AE*.

This set of experiments, then, corroborates in a clear-cut manner the general result derived from all the previous ones; that is, general environmental changes, especially if they are pronounced, usually bring on endomixis slightly earlier than it would have occurred if the cells had been left in the environment in which they were at the last reorganization period. But after this initial change the periodicity characteristic of the organism is resumed and persists.

In addition, however, this experiment suggests another point of interest: the length of the rhythm is apparently partially independent of the number of cell divisions—the periodicity being one in which time, so to speak, is an important factor. This is a most surprising result, because such profound reorganization phenomena as are involved in endomixis must bear a more or less definite relation to the physiological activity of the protoplasm, the best criterion of which is generally considered to be growth and reproduction as indicated by the division rate. More experiments obviously are needed to resolve this 'time factor' into its significant elements.

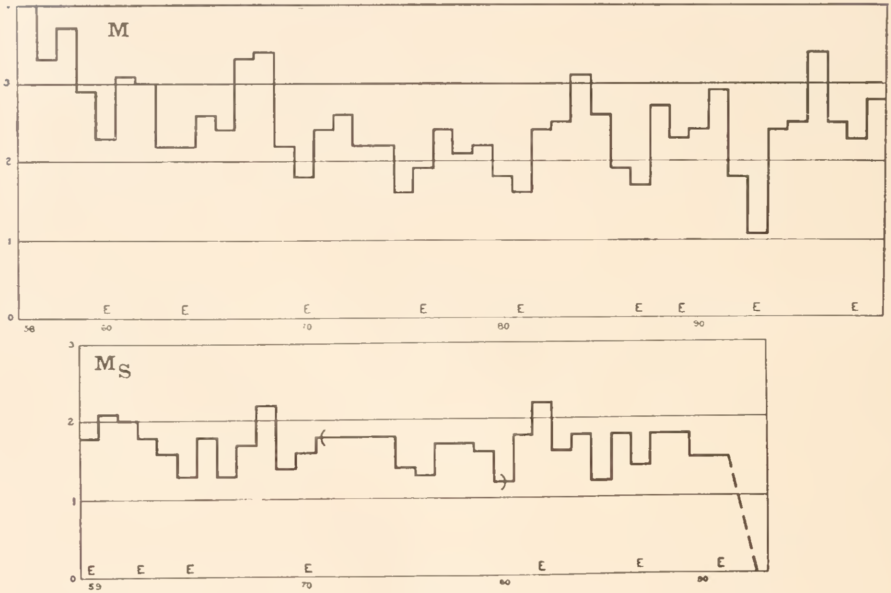

FIG. 12.

EXPERIMENTS—SERIES III.

Peebles states that Horlick's Malted Milk is a most satisfactory culture medium for *Paramecium* if used in a .2 per cent.

solution,[1] and therefore this seemed to offer an opportunity to study the effect of a medium radically different in composition from those previously employed in these studies. Accordingly subcultures designated *Am*1, *Am*2, *Am*3, *AEm*1, *AEm*2, *Om* and *Mm* were started from *A*, *AE*, *O* and *M* respectively, and bred on this medium. The extent of these cultures and the time of appearance of endomixis (*E*) in the two sets is shown in the following table:

Period	52	53	54	55	56	57	58	59	60
A		*E*				*E*			
*Am*1	(from *A*)	*E*				*E*			
*Am*2	(" *A*)	*E*				*E*			
*Am*3						(from *A*)	*E*		
AE					*E*			*E*	
*AEm*1	(from *AE*)	*E*			*E*		discontinued		
*AEm*2						(from *AE*)	*E*		
O						*E*			
Om	(from *O*)					*E*			
M									*E*
Mm						(from *M*)	*E*		

This table shows that the malted milk medium did not change at all the periodicity of endomixis in either the *A* or *O* milk subcultures, while it consistently brought it about earlier in the *AE* and *M* milk series. The results from the *AE* and *M* cultures are therefore in accord with the results obtained in Series II of these experiments, while those with *A* and *O* apparently differ. However, the non-appearance of endomixis earlier in the *Am* series is readily explained by the fact that the process was just about to occur in the parent culture when the *Am* subcultures were branched, as is shown by the fact that it appeared in *A* during the following period.

The experiments with malted milk are obviously not extensive enough to give any details of the effect of long subjection to this medium on endomictic periodicity, but they adequately answer, it is believed, the purpose of the present study by again indicating that the appearance of endomixis may be slightly altered temporarily by subjecting *Paramecium* to a markedly changed environment.

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[1] Peebles, "Regeneration and Regulation in *Paramecium caudatum*," BIOL. BULL., 1912.

EXPERIMENTS—SERIES IV.

This series is a brief repetition of Series I., since it comprises a comparison of the periodicity of endomixis both in the same races when bred under varied and constant culture conditions, and in different races bred under varied and constant culture conditions. The cultures employed were *A*, *AE* and *B* which were used in the first series, and also two other cultures, *M* and *W*.

The following table shows a practically perfect synchronism of endomixis in all the races under the different environmental conditions:

Period.........	81	82	83	86	87	88	89	90
A............		*E*		*E*				*E*
At2...........			(from *A*)	*E*				*E*
AE...........		*E*		*E*				*E*
AEt2.........			(from *AE*)	*E*				*E*
B............	*E*				*E*			*E*
Bt2...........			(from *B*)	*E*				*E*
M............	*E*				*E*		*E*	
Mt2..........			(from *M*)	*E*				*E*
W............	*E*			*E*				
Wt2..........			(from *W*)	*E*				

This is exactly the same result which was obtained in the experiments of Series I. In Series I this synchronism was most satisfactorily accounted for by assuming that there was an initial effect on the periodicity by the change of environmental conditions to which all the cultures were subjected at the start. That this assumption was justified is shown by experiment *C*, Series II (cf. p. 455). However, it is to be noted in the present experiment that there is no apparent initial influence of the changed culture conditions, but this is probably due to the fact that the *A*, *AE*, *B*, *M* and *W* cultures had been so long under the same environmental conditions before their respective *t*2 subcultures were branched from them. Consequently the synchronism of the *t2* set is due merely to maintaining the periodicity of the respective parent cultures.

GENERAL SUMMARY.

All four series of experiments show that the general 'time-periodicity' of rhythms and endomixis in *Paramecium aurelia* is the same in the several races which have been studied under the following environmental conditions:

1. Varied culture medium changed daily, and at room temperatures.
2. Varied culture medium changed on alternate days, and at room temperatures.
3. Constant beef extract culture medium and at a temperature of 26° C.
4. Horlick's malted milk medium, and at room temperatures.

Thus it seems clear that one question which this study was planned to elucidate has been answered: General changes in the environment of the animals, as markedly different culture media and temperatures, such as may be termed normal changes, do not permanently modify the length of the rhythm or the time between successive endomictic periods which is characteristic of the species.

However, sudden marked changes in normal culture conditions may initially induce the appearance of the definitive endomictic phenomena slightly earlier than they would have occurred if the cell had been continued under its former environmental conditions; but this initial disturbance is soon compensated for, usually within the present rhythm, so that the previous characteristic periodicity is again resumed.

Throughout all the work there is evident a remarkable *synchronism* of the endomictic process in all the races bred simultaneously, regardless of the environmental conditions. Thus not only is the periodicity of endomixis, or length of the rhythm, the same, as stated above, but also the rhythmic periods are synchronous. The explanation of this is clearly due, in the experiments involving the most marked changes in the cultural conditions, to an initial effect of these changes, which brings into line, so to speak, the appearance of endomixis in all the cultures.

Consequently it is highly probable that a slight initial shift of the definitive onset of endomixis in the various races is the explanation of the nearly simultaneous appearance of the process in all the races under all the conditions.

Although the 'time-periodicity' characteristic of the species has been shown by the present experiments to be practically unmodifiable under the general environmental changes which were employed, it has been found that the 'generation-periodicity'—or the number of cell divisions between one occurrence of endomixis and the next—may be modified to a considerable degree by the culture conditions which lower the division rate. In other words, the rhythm appears to be more susceptible of modification in regard to generations than time. As has been previously noted, this is a surprising result, since a profound reorganization process such as endomixis must be closely related to the general metabolism of the cell and this is expressed to a large extent in growth and reproduction. Further work on this problem is in progress.

Finally, the cessation of endomixis in these experiments was invariably followed, usually within a rhythm or two, by the death of the culture involved. This indicates strongly, if it does not prove, that a periodic occurrence of the definitive endomictic phenomena is a *sine qua non* for the continued life of the race—a conclusion which is concordant with all previous data in regard to endomixis.

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